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(54) **METHOD FOR INDUCING AN IMMUNE RESPONSE FOR TREATMENT OF CANCER AND AUTOIMMUNE DISEASES OR CONDITIONS**

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(58) **Field of Classification Search**
None
See application file for complete search history.

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(57)   **ABSTRACT**

The invention is related to peptide constructs, i.e., polypeptides obtained by linking together two or more peptides based on or derived from different molecules, which are useful in the treatment or prevention of cancer or the treatment of autoimmune diseases and compositions containing same, methods for producing same, and methods for using same; wherein the peptide constructs have the formula P_1-x-P_2 where P_2 is a peptide associated with forms of cancer or an autoimmune condition and P_1 is a peptide which will bind to a class of immune cells such as dendritic cells. The peptide construct can cause the maturation of immature dendritic cells to a more mature state. The peptide construct or the more mature dendritic cells can be administered to a subject to a modulate or to initiate an immune response against cancer cells, and can be used with dyes, radioisotopes, or therapeutic agents for detection of the immune target and/or treatment of cancer and autoimmune conditions.

22 Claims, 4 Drawing Sheets

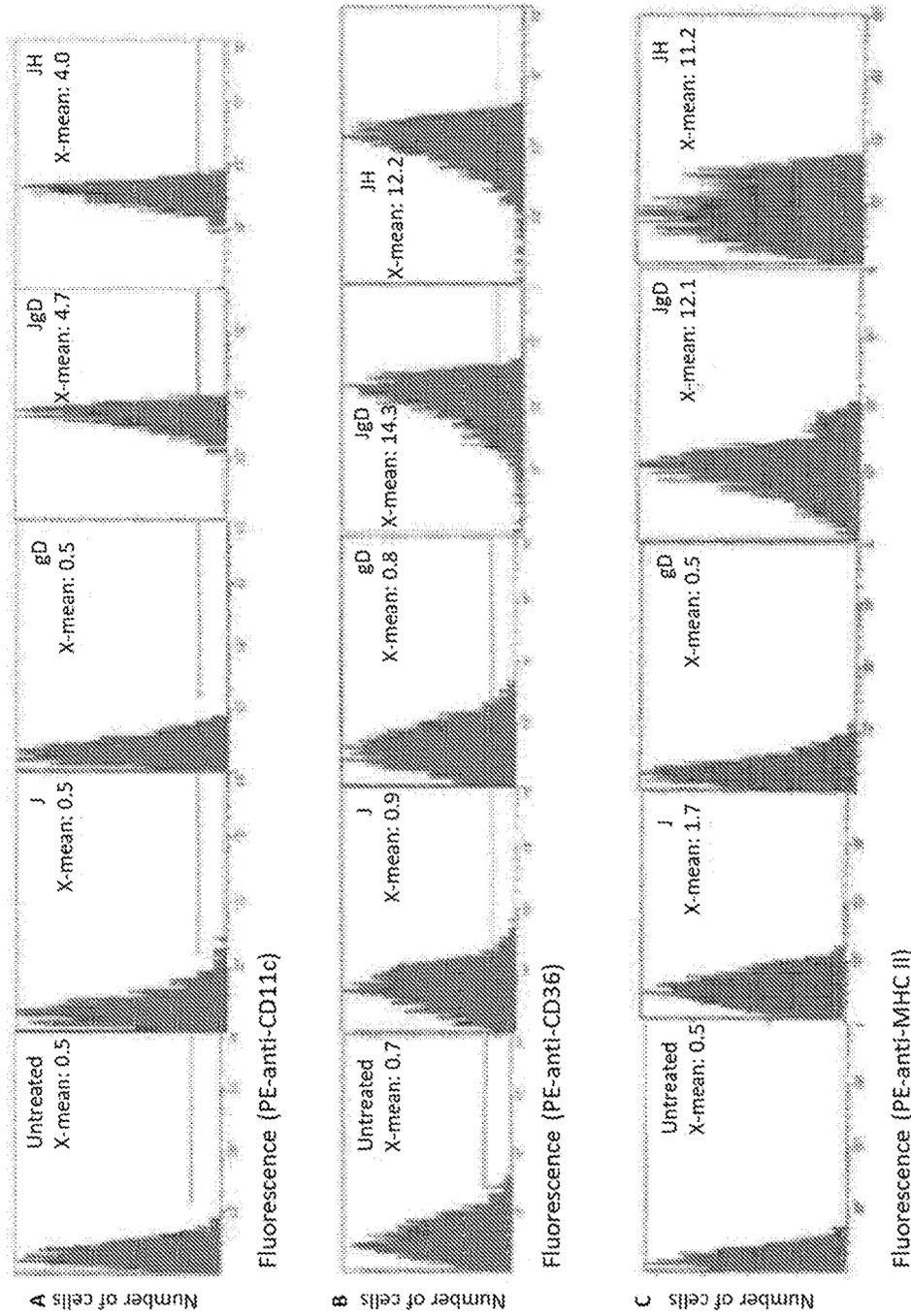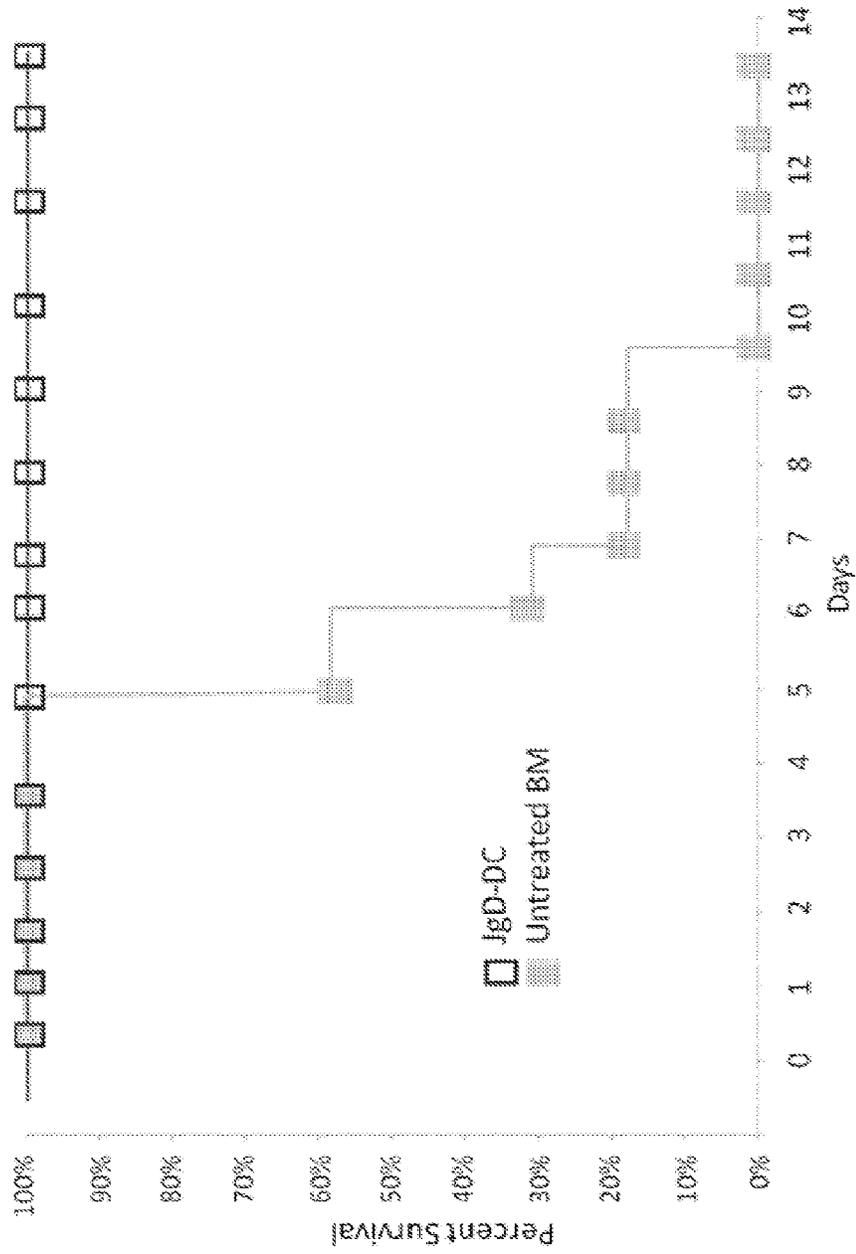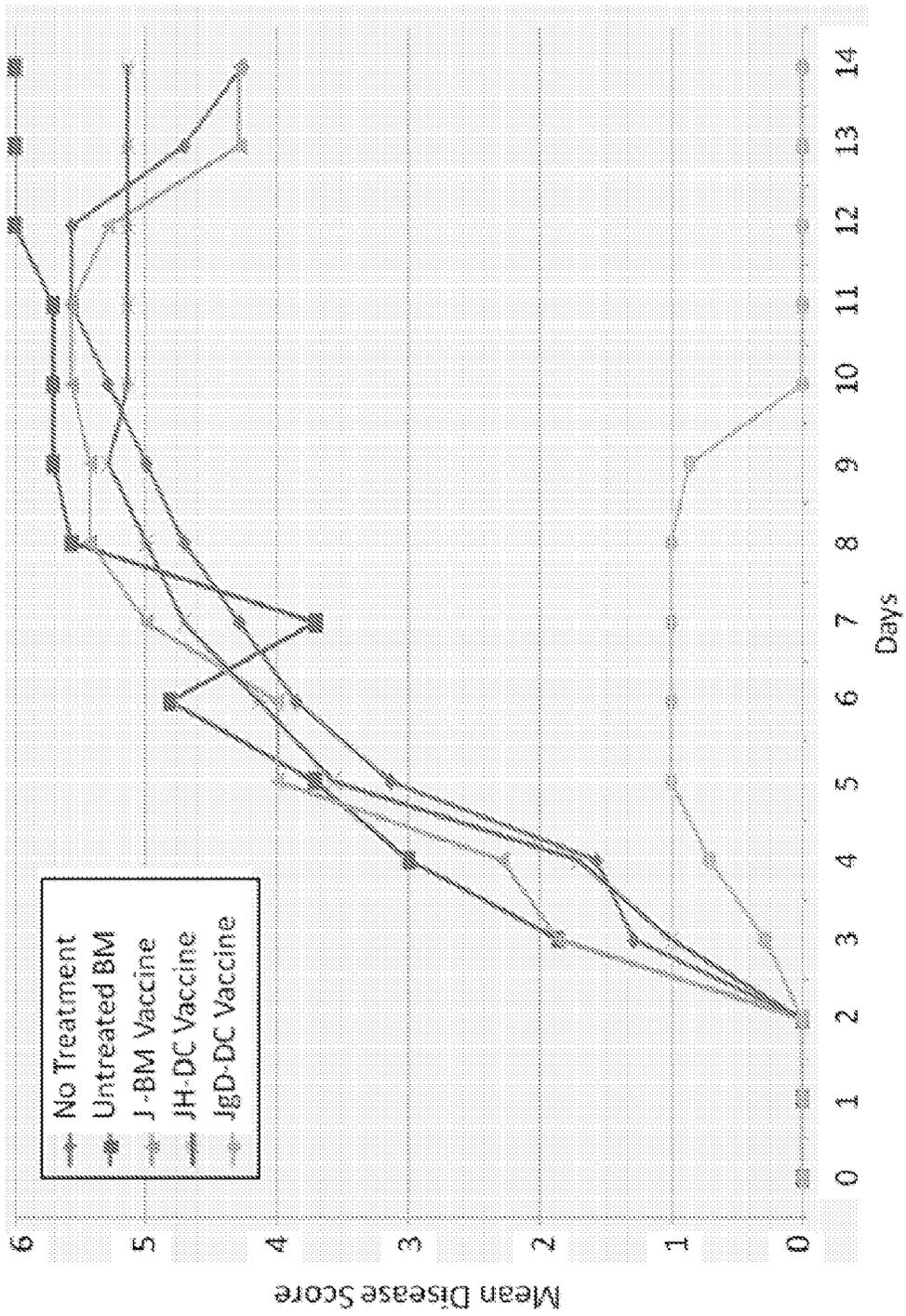**Specification includes a Sequence Listing.**

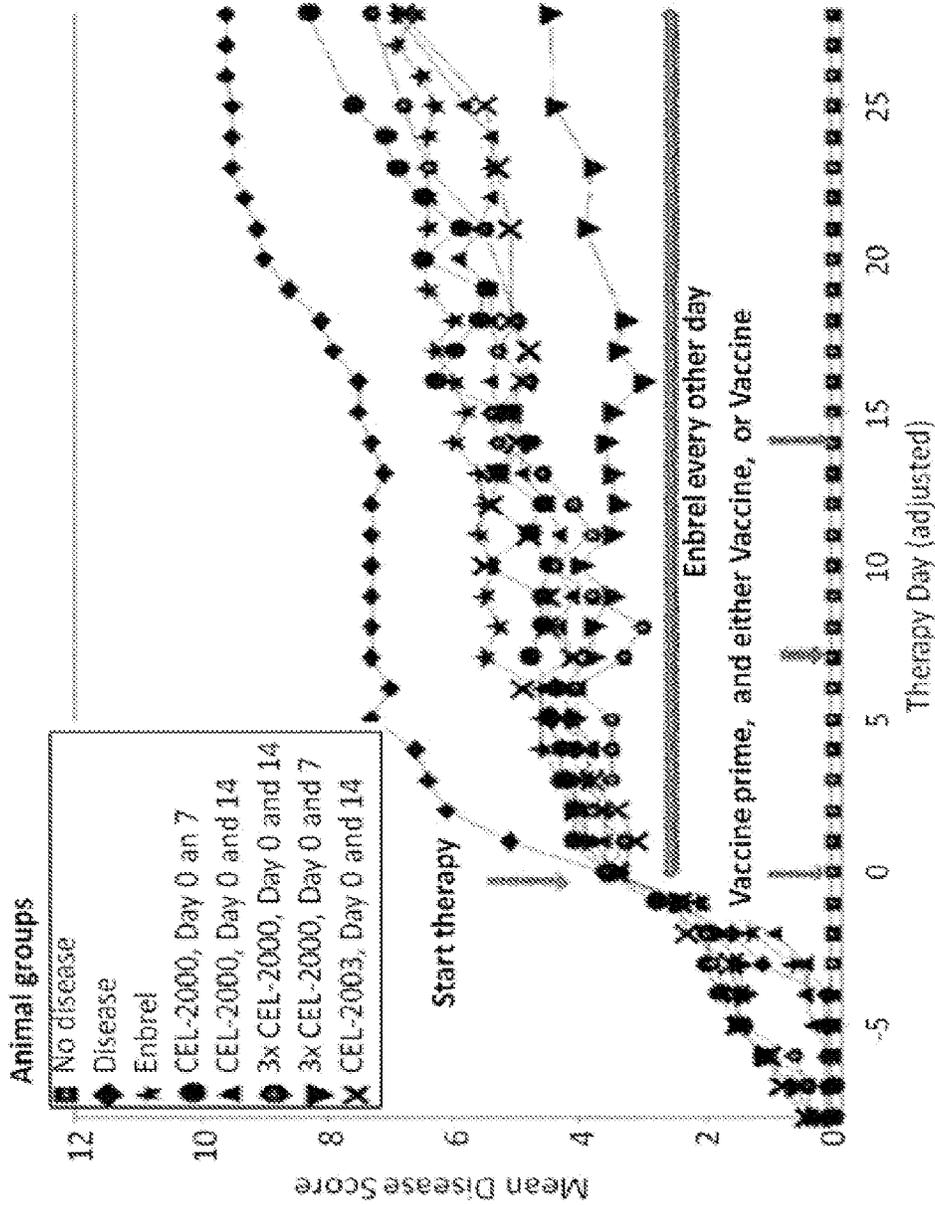**Figure 1**

Figure 2

Figure 3

Figure 4

METHOD FOR INDUCING AN IMMUNE RESPONSE FOR TREATMENT OF CANCER AND AUTOIMMUNE DISEASES OR CONDITIONS

SEQUENCE LISTING

This application contains a "Sequence Listing" submitted as an electronic .txt file named "CS_ST25.txt." The subject matter of the "Sequence Listing" is incorporated herein by reference along with the subject matter of U.S. patent application Ser. Nos. 12/992,687, 11/443,314, 61/489,926 and 61/489,986, U.S. Pat. Nos. 6,995,237 and 7,256,254, and International Published Patent Application WO 2010/120897 A1 (PCT/US2010/031054).

FIELD OF INVENTION

The invention generally related to methods and compositions for activating and promoting the maturation of immature dendritic cells or monocytes into matured dendritic cells (DCs) and eliciting favorable properties in the matured dendritic cells.

BACKGROUND

Autoimmune conditions are characterized by the body attacking itself by mounting an immune response against self antigens to which it is normally tolerant. As such, approaches to treating autoimmune conditions, have focused on down regulating the "inappropriate" immune response against self. However, many approaches to treating autoimmune and like conditions are not specific to down regulating the immune system's response to a specific antigen. Rather, therapies focus on a general suppression of the immune system. Similarly, cancer can be characterized by increased expression of self oncogenes that are not adequately recognized by the immune system. As such, adjustment of the regulation of the immune system in regards to the identification of self antigens can be used to address both autoimmune conditions and cancer.

Some of these less antigen specific approaches utilize monoclonal antibodies that act on activated T cells and down regulate them such as by anti-CD3 (Protein Design Laboratories) or block APC and T cell interaction by anti-ICAM-3 (ICOS). MEDI-507 (Medimmune) is believed to be a humanized monoclonal antibody, for psoriasis that also targets CD2, presumably for removing or inactivating those cell types. Other diseases, such as, tissue transplantation rejection and allergies are also being tested by this approach. In contrast to acting on cell surface markers, rhu-mAB-E25 (Genentech) is believed to be a humanized monoclonal antibody against IgE that binds to circulating IgE, with the goal of preventing activation of mast cells. In contrast, other researchers are developing monoclonal antibodies to act on symptoms or agents directly causing disease symptoms. Remicade Infliximab (Centocor™) is purported to be a monoclonal antibody to TNF. Anti CD40 ligand has been used for treatment in animal model of multiple sclerosis (MS) (L. M. Howard, et al., 1999, J. Clin. Invest, 103:281). A recombinant generated designed protein Enbrel (Immunex™) is purported to comprise two molecules of r-DNA derived TNF receptor, and is intended to block TNF's action.

It should be noted, however, that many of these agents are not sufficiently disease specific and often recognize and

could affect normal cellular and body constituents that have a defined and necessary role in normal immune defenses which are still needed.

Some more antigen or disease specific approaches are exemplified by the attempt to treat MS patients by oral administration of myelin proteins which have recently been reported; the same group of researchers is also using collagen type II for treatment of patients with rheumatoid arthritis. These treatments are designed to attack at the level of the gut associated lymphoid tissues (GALT) to induce tolerance by antigen specific suppression of the immune system. It is not know if these treatments use the intact protein or a hydrolyzate containing smaller peptides. See D. Hafler, et al. 1988, J. Immunol., 141:181; K. Wucherpfennig, et. al. 1990, Science, 248:1016; K. Ota, et al., 1990, Nature, 346:183; and H. Weiner, 1999, PNAS, 88:9161.

Several researchers are testing peptide based materials for treatment of autoimmune conditions. One approach uses peptide as immunogen, given orally in large quantities. The peptide represents a peptide sequence that is though to be the autoimmune epitope itself or a modified form which may also have altered binding or improved stability properties. By use of the peptide it is thought that either the normal peptide or an altered peptide ligand (APL) will bind to the T cell receptor (TCR) and induce a state of anergy since the multiple sets of bindings that would occur with antigen presentation with an antigen presenting cell (APC) do not occur (A. Faith, et al., 1999, J. Immunol., 162:1836; Soares, et al. 1998, J. Immunol., 160:4768; M. Croft, et al. 1997, J. Immunol., 159:3257; L. Ding, et al., 1998, J. Immunol., 161:6614; and S. Hin, et al. 1999, J. Immunol., 163:2363). Some of the approaches with APL include using related amino acids such a D amino acids (U. Koch, et al. 1998, J. Immunol., 161:421), amino acids with substituted side chains (R. DePalma et al. 1999, J. Immunol., 162:1982), methylene groups to replace peptide bonds in the peptide backbone (L. Meda, et al., 1996, J. Immunol., 157:1213) and N-hydroxyl peptides (S. Hin et al. J. Immunol., 163:2363).

The more antigen-specific approaches outlined above rely on using large amounts of antigens to desensitize a subject to the antigen. The possible drawbacks and consequences of the administration of large quantities of antigen include further undesirable and unpredictable immune responses. Peptide-based immunomodulators have the possible advantage of being a well-defined immunogen that would facilitate the generation of a safe and predictable response. However, safety is maximized by the use of small quantities of immunomodulators targeted to specific immune cells instead of the use of large quantities of an antigen introduced into the patient.

Few therapeutics are available as recombinant proteins that can modulate the immune system in active and antigen-specific capacity. Viral vector vaccines have been attempted to promote antigen-specific immunomodulation. However, problems are also associated with viral vector vaccines. One problem is the immune response induced against the vector itself. This induced immune response severely limits the number and frequency of subsequent injections/boosters that can be administered. Moreover, some adenoviruses have the potential for causing allergic conditions such as celiac disease. It is also known that many viral proteins, including some from HIV and HSV contain immunosuppressive epitopes. Viral proteins are also suspected as causative agents for other autoimmune conditions such as type 1 diabetes. Multiple Sclerosis (MS), Myocarditis, and Graves disease.

Another disadvantage in using a DNA-based or viral vector vaccines, including for autoimmune conditions, is the possibility of the vaccine DNA being integrated into the host's genome. One alternative is to conjugate a particular epitope to a carrier protein to avoid such incorporation into the genome. It is known within the art to use large carrier proteins such as Keyhole Limpet Hemocyanin (KLH), Bovine Serum Albumin (BSA), or Antigenics' heat shock proteins (HSP) couple/conjugated or incorporated with a virus protein.

Other options for peptide delivery of peptide epitopes include the use of synthetic biodegradable microparticles like Poly(lactide-co-glycolide) PLG with aggregated antigen. Still other delivery technologies for peptide antigens include AutoVac™ of Pharmexa. Other small molecule delivery technologies for peptides are Antigen Express's 'li-key' delivery, phage display and Multiple Antigen Presentation (MAPS) technologies (Rosenthal 2005 Immune peptide enhancement of peptide based vaccines Frontiers in Bioscience 1:478:482).

Many of the known approaches have the major disadvantage of using large, very immunogenic carriers. Moreover, patient populations requiring such therapeutics have usually been exposed to many of these same antigens during their lifetimes. Hence, and similar to vaccine vector delivery of antigens, clearance of the antigen can be so vigorous in the previously exposed host that no response will occur to the new antigen. On the other hand, a strong immune response may occur upon reintroduction of the vector. For example, in the case of the conjugate VP22 containing HSV-1 protein, the response may be undesirable given that a majority of adults have had one or more exposures to HSV-1 (Muranyiova et al. 1991 Immunoprecipitation of herpes simplex virus polypeptides with human sera is related to their ELISA titre. Acta Virol, 35:252-9).

In regards to immune-based cancer therapies, cancer can have many causes that result in the uncontrolled growth of cells. One cause of cancers is the mutation or increased expression of oncogenes in cancerous cells. Oncogenes, like all genes, function to code for a protein that is synthesized by the cell. Often, cancers are caused by a DNA mutation that alters the regulation of expression of the oncogene or oncofetal gene or a DNA mutation that causes a change in the amino acid sequence of the oncogene itself. The alteration of regulation of an oncogene or oncofetal gene or a mutation in an oncogene or oncofetal can also affect the expression of other proteins in a cancerous cell. Cancerous cells can, therefore, have different levels of protein expression compared with surrounding healthy tissue. A change in the level of protein expression or the expression of mutated proteins on the surface of cancerous can be used by the immune system to direct an immune response to cancerous cells.

The proteins expressed on the surface of a cancerous cell can act as antigens for an immune response. However, most of the proteins expressed on the surface of cancerous cells are non-mutated self-antigens. Self antigens are usually ineffective in triggering an immune response since they are present in healthy as well as cancerous cells. Even in situations where a cancer cell is expressing a mutated protein, antigenic changes in cancerous cells that are created by individual point mutations may be too subtle from the standpoint of the immune system to trigger a significant immune response. Since cancer cells utilize essentially the same cellular proteins as healthy cells, cancer cells can often grow and survive without generating an anti-cancer immune response.

Peptides can have sufficient structure to be recognized with specificity by immunoproteins, such as antibodies, and by immune cells. That is, short peptides having from about 8 to about 30 amino acid residues have sufficient structure to bind to antibodies and serve as an antigen, epitope or other ligand for proteins involved in the activation of the immune system. However, short peptides often generate no or only a weak immune response when administered alone to a human or animal subject. Often, it is necessary to link or to introduce short peptides with larger proteins or biomolecules to serve as a carrier or an adjuvant to induce an immune response that will generate antibodies specific to the short peptide and to initiate an immune response to these short peptides.

There is a need for peptide-based immunomodulators having a well-defined immunogen to treat cancer that facilitates the generation of a safe and predictable anti-tumor response rather than a mixed response including an immune response to a carrier. There is also a need for the development of peptide-based immunomodulators, and the related need for the identification of peptides capable of being recognized by specific components of the immune systems and generating a specific type of directed immune response.

SUMMARY OF THE INVENTION

The peptide constructs disclosed herein are based on a Ligand Epitope Antigen Presentation System (LEAPS™) which can convert small peptides, which typically do not exhibit a strong effect on the immune system into antigen specific immunomodulators. The immunomodulators disclosed herein have the ability to promote the differentiation of immature dendritic cells (DCs) to matured DCs that are educated or competent to affect other components of the immune system with respect to specific antigens. The immunogens disclosed herein promote the upregulation of CD11c, CD86 and Major Histocompatibility Complex class II (MHC II) in immature DCs isolated or generated from bone marrow cells, which are phenotypical indications of matured DCs. The matured DCs have an increased production of IL-12, particularly IL-12p70, indicated matured DCs that are competent to signal a TH1-type immune response.

Upon administration to a subject, DCs matured with the immunomodulator disclosed herein have the capability to locate to a site in the subjects body harboring a source of the antigen to which an autoimmune response is directed. In certain embodiments, the administered matured DCs have the ability to modulate an immune response to an autoimmune antigen.

In certain embodiments, DCs matured with the immunomodulators disclosed herein have an ability to locate or "target" to a site in the subject's body harboring a source of the antigen and/or the site of an autoimmune condition. The DCs matured with the immunomodulators can be used to diagnose or determine the presence or location in the body of an autoimmune response in a subject to which the matured DCs are administered. The immunomodulators can be conjugated with a radionuclide (including ^{18}F, ^{32}P, ^{64}Cu, ^{90}Y, ^{99m}Tc, ^{131}I, ^{125}I, ^{124}I, ^{80}Zr, ^{111}In, ^{188}Re, or ^{177}Lu) that co-locate with the matured DCs. The location of the matured DCs can then be determined through appropriate radiation-detection techniques to diagnose the presence and/or location of an autoimmune condition in the body of the subject.

In certain embodiments, the matured DCs are conjugated or associated with a radionuclide (including ^{18}F, ^{32}P, ^{64}Cu, ^{90}Y, ^{99m}Tc, ^{131}I, ^{125}I, ^{124}I, ^{89}Zr, ^{111}In, ^{188}Re, or ^{177}Lu) that locates to the location of an autoimmune or other undesir-

able immune response in the body of a subject to which the matured DCs are administered. The radionucleotide can be conjugated to an antibody (mAb) to CD11c or MHC II located on the surface of the matured DCs. In the alternative, an antibody can have specificity to other cell surface markers including DEC-205, Dectin-1, DC-SIGN, and DC-LAMP. In certain embodiments, an mAb binds to markers on the surface of DCs such that the binding of the mAb to the DCs does not alter the activation or changes induced by the peptide construct heteroconjugates described herein.

In certain embodiments, radiation from radioisotopes can be used for detection by X-ray sensitive films or instruments or related technologies. The compositions of the invention can be detected by single-photon emission tomography/computed tomography (SPECT/CT), and ^{99}Tc, ^{201}Ti and ^{89}Zr can be used to generate photon emission tomography (PET) images. Sources of radiation can be conjugated to the immunomodulator peptide used to mature the DCs or can be conjugated to an mAb having specificity for the matured DCs.

In certain embodiments, the matured DCs can be conjugated or associated with a therapeutic agent. The therapeutic agent can co-locate to the site of an autoimmune or other undesirable immune response together with matured DCs administered to a subject.

In certain embodiments, the matured DCs can be conjugated or associated with a dye agent. The dye agent can co-locate to the site of an autoimmune or other undesirable immune response to allow for the diagnostic detection or imaging of the site.

In certain embodiments, a composition comprising a population of matured dendritic cells is provided. The population of matured dendritic cells is formed by treating immature dendritic cells of monocytes with an effective amount of a peptide construct serving as an immunomodulator having the formula P_1-x-P_2 or P_2-x-P_1 under conditions suitable for maturation of the cells to form the matured or effective dendritic cells which interacts with T cells, where P_2 represents a specific antigenic peptide derived from an autoimmune antigen, P_1 represents an immunomodulatory peptide which is a portion of an immunoprotein capable of promoting binding to a class or subclass of DC cells, and -x- represents a covalent bond or a divalent linking group.

In certain embodiments, a method for targeting matured or effective dendritic cells to a site of an autoimmune condition in a subject is provided. Immature dendritic cells or monocytes are treated with a peptide construct immunomodulator ex vivo under conditions suitable for maturation of the cells to form more matured or activated dendritic cells, and an effective amount of these matured dendritic cells are administered to the subject, wherein a majority of the dendritic cells administered to the subject locate to the site of an autoimmune or other undesirable immune response.

Upon administration to a subject, DCs matured ex vivo with the immunogens disclosed herein have the capability to locate to a site in the subjects body harboring a source of the antigen contained in the immunogen such as a cancer tumor, a cluster of cancer cells or any other antigen source. In certain embodiments, the administered matured DCs have the ability to direct an immune response against a source of the antigen, such as cancer cells, such that the subject's immune system can be modulated to kill cancer cells.

In certain embodiments, DCs matured with the immunogens disclosed herein have an ability to locate or "target" to a site in the subject's body harboring a source of the antigen contained in the immunogen such as a cancer tumor, a

cluster of cancer cells or any other antigen source. The ability of DCs matured with the immunogens can be used to diagnose or determine the presence of cancer cells or other sources of antigens in the body of a subject to which the matured DCs are administered. The immunogens can be conjugated with a therapeutic agent or a radionuclide (including ^{18}P, ^{32}P, ^{64}Cu, ^{90}Y, ^{99m}Tc, ^{131}I, ^{125}I, ^{124}I, ^{111}In, ^{188}Re, or ^{177}Lu) that co-locate the matured DCs. The location of the matured DCs can then be determined through appropriate radiation-detection techniques to diagnose the presence and/or location of cancer cells or other sources of antigens in the body of the subject.

In certain embodiments, the matured DCs are conjugated or associated with a radionuclide (including ^{18}F, ^{32}P, ^{64}Cu, ^{90}Y, ^{99m}Tc, ^{131}I, ^{125}I, ^{124}I, ^{89}Zr, ^{11}In, ^{188}Re, or ^{177}Lu) that locates to the location of cancer cells or other sources of antigens in the body of a subject to which the matured DCs are administered. The radionuclide can be conjugated to an antibody (Mab) to CD11c or CD3 located on the surface of the matured DCs.

In certain embodiments, the matured DCs is conjugated or associated with a radionuclide that is a source of ionizing radiation. Examples of sources of ionizing radiation include high-energy β-emitters, such as certain isotopes of yttrium or rhenium, α-emitters or position emission such as ^{64}Cu, ^{124}I and certain isotopes of bismuth or astatine. Matured DCs conjugated or associated with sources of ionizing radiation are used to deliver the ionization radiation primarily at the site of cancer cells or other source of antigen where the antigen is being expressed due to the location of the matured DCs to the site cancer cells or other sources of antigen. High-energy and short half-life gamma emitters can be used for detection of X-ray sensitive films or instruments or related technologies. ^{111}In can be detected by single-photon emission tomography/computed tomography (SPECT/CT), and ^{99}Te, ^{201}Ti and ^{89}Zr can be used to generate photon emission tomography (PET) images. Sources of ionizing radiation can be conjugated to immunogen used to mature the DCs or can be conjugated to an mAb having specificity for the matured DCs.

In certain embodiments, the matured DCs are conjugated or associated with a therapeutic agent which can be cytotoxic or other drug or toxin or cytokine with preferential toxicity to cancerous cells. The therapeutic agent can co-locate to the site cancer cells or other sources of antigen together with matured DCs administered to a subject.

In certain embodiments, the matured DCs are conjugated or associated with a dye (e.g., fluorescent or luminescent) agent. The dye agent can co-locate to the site of cancer cells or other sources of antigen together with matured DCs administered to a subject and allow for the diagnostic detection or imaging of cancer cells or other sources of antigen.

In certain embodiments, the LEAPS matured DCs are conjugated or associated with a monoclonal antibody or lectin which reacts or binds with the surface of the DC (such as CD11c, MHC II or other potential DC markers such as DEC-205. Dectin-1, DC-SIGN, DC-LAMP) in a manner that does not alter the response on the LEAPS activated DC and further this Monoclonal antibody can be conjugated or associated with a radioactive nucleotide, drug (cytotoxic drug or other cancer treating drug), toxin, cytokine or staphylococcus endotoxin A or B (SHE or SEB) or a dye (e.g., fluorescent or luminescent) agent.

In certain embodiments, a composition has a population of matured dendritic cells is provided. The population of matured dendritic cells is formed by treating immature

dendritic cells or monocytes with an effective amount of a peptide construct having the formula P_1-x-P_2 or P_2-x-P_1 under conditions suitable for maturation of the cells to form the matured or effective dendritic cells which interacts with T cells, where P_2 represents a specific antigenic peptide derived from a cancer cell. P_1 represents an immunomodulatory peptide which is a portion of an immunoprotein capable of promoting binding to a class or subclass of DC cells, and -x- represents a covalent bond or a divalent linking group.

A method for targeting matured or effective dendritic cells to a site of cancer cells in a subject is provided. Immature dendritic cells of monocytes are treated with a peptide construct ex vivo under conditions suitable for maturation of the cells to form more matured or activated dendritic cells, and an effective amount of these matured dendritic cells are administered to the subject, wherein a majority of the dendritic cells administered to the subject locate to the site of cancer cells.

In certain embodiments, a peptide for directing an immune response in an autoimmune condition or to cancer or for maturing dendritic cells is a peptide construct selected from the group consisting of SEQ ID No.'s 291, 293, 315, 317, 319, 325, 331, 339, 341, 347, 355, 782, 786, 804, 828, 856-866, 867-879, 881, 883-884, 886-895, 897, 899-904, 906-916, 964-996, and 1045-1090 or a variant thereof.

In certain embodiments, a composition containing matured dendritic cells is provided. The matured dendritic cells are formed by contacting immature dendritic cells or monocytes with an effective amount of a peptide construct having the formula P_1-x-P_2 or P_2-x-P_1 under conditions suitable for maturation of the immature dendritic cells to form the matured dendritic cells, wherein P_2 represents a peptide derived from a cancer cell or derived from a protein involved in an autoimmune disease competent for recognition by a class or subclass or immune cells or binding to an antibody: P_1 represents an immunomodulatory peptide which is a portion of an immunoprotein capable of promoting binding to a class or subclass of dendritic cells; and x represents a covalent bond or a divalent linking group.

In certain embodiments, a composition containing a population of matured dendritic cells is provided. The matured dendritic cells formed are by contacting immature dendritic cells or monocytes with an effective amount of a peptide construct selected from the group consisting of SEQ ID No.'s 291, 293, 315, 317, 319, 325, 331, 339, 341, 347, 355, 782, 786, 804, 828, 856-866, 867-879, 881, 883-884, 886-895, 897, 899-904, 906-916, 964-966, and 1045-1090 or a variant thereof under conditions suitable for maturation of the dendritic cells of monocytes.

In certain embodiments, a method for modulating an immune response in an autoimmune disease or condition or to cancer includes administering an immunologically effective amount of a peptide construct selected from the group consisting of SEQ ID No.'s 291, 293, 315, 317, 319, 325, 331, 339, 341, 347, 355, 782, 786, 804, 828, 856-866, 867-879, 881, 883-884, 886-895, 897, 899-904, 906-916, 964-966, and 1045-1090 or a variant thereof or a variant thereof to a subject.

In certain embodiments, a method for producing a matured dendritic cell population is performed by contacting or treating immature dendritic cells or monocytes with an effective amount of a peptide construct having the formula P_1—X—P_2 or P_2—X—P_1 under conditions suitable for maturation of dendritic cells or monocytes to form matured dendritic cells, wherein P_2 represents a peptide derived from a cancer cell or derived from a protein involved in an

autoimmune disease competent for recognition by a class or subclass of immune cells or binding to an antibody: P_1 represents an immunomodulatory peptide which is a protein of an immunoprotein capable of promoting binding to a class or subclass of dendritic cells; and x represents a covalent bond or a divalent peptide linking group.

In certain embodiments, a tracking marker or a therapeutic agent is conjugated to an antibody having affinity for any one of MHC II, CD11c, DEC-205, Dectin-1, DC-SIGN, and DC-LAMP.

In certain embodiments, matured dendritic cells exhibit an upregulation of one or more of CD80, CD86 and Major Histocompatibility Complex II relative to immature dendritic cells or monocytes not contacted with the peptide construct.

In certain embodiments, matured dendritic cells are isolated away from bone marrow or blood tissues.

In certain embodiments, matured dendritic cells produce an increased amount of Interleukin 12p70 (IL-12p70) compared to immature dendritic cells or monocytes not contacted with the peptide construct.

In certain embodiments, a therapeutic agent or a tracking marker is conjugated to a peptide conjugate the peptide construct is conjugated to the therapeutic agent by a cathepsin cleavable valine-citrulline dipeptide linker or by linking with a cysteine or lysine residue of the peptide construct by conjugation to a group selected from OH groups, COOH groups, amine groups, and amide groups of the peptide construct.

In certain embodiments, a peptide construct is conjugated to a lysomatropic agent.

In certain embodiments, an immune response in a subject is modulated in response to an autoimmune disease or condition or against cancer by contracting immature dendritic cells or monocytes with a peptide construct having the formula P_1-x-P_2 or P_2-x-P_1 under conditions suitable for maturation of the cells to form matured dendritic cells and administering an effective amount of the matured dendritic cells to the subject. In the peptide construct, P_2 represents a peptide derived from a cancer cell or derived from a protein involved in an autoimmune disease competent for recognition by a class or subclass of immune cells or binding to an antibody: P_1 represents an immunomodulatory peptide which is a portion of an immunoprotein capable of promoting binding to a class or subclass of dendritic cells; and x represents a covalent bond or a divalent peptide linking group.

In certain embodiments, the peptide construct having the formula P_1-x-P_2 or P_2-x-P_1 has a peptide P_1 selected from the group consisting of SEQ ID No.'s 4-6, 13, 15, 27, 48 and 49 or variants thereof.

In certain embodiments, the peptide construct having the formula P_1-x-P_2 has a peptide P_2 selected from one of the following groups; the group consisting of SEQ ID No.'s 51, 53, 55 and 960-961; SEQ ID No.'s SEQ ID No.'s 51, 53, 55 and 960-961; the group consisting of SEQ ID No.'s 72, 749, 751, 753, 755 and 917; the group consisting of SEQ ID No.'s 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 98, 103, 449, 452, 469, 918, 957 and 958; the group consisting of SEQ ID No.'s 1, 474, 477, 482, 487, 490, 493 and 812 and 962-963; SEQ ID No.'s 496, 499, 919, 920, 921, and 922; the group consisting of SEQ ID No.'s 2, 524, 527, 549, 552, 555, 572, 581, 588, 597, 606, 609, 612, 623, 636, 641, 924, 925, 950 and 959; SEQ ID No.'s 653 and 664; the group consisting of SEQ ID No.'s 3 and 927; SEQ ID No. 774; the group consisting of SEQ ID No.'s 19, 737, 738, 951; SEQ ID No.'s 928 and 947; the group consisting of SEQ ID No.'s 929 and 948; the

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group consisting of SEQ ID No.'s 930936 and 949; the group consisting of SEQ ID No.'s 937-940; the group consisting of SEQ ID No.'s 941-942; the group consisting of SEQ ID No.'s 943-944; and the group consisting of SEQ ID No.'s 945-946, or variants of any of the foregoing sequences.

In certain embodiments, the peptide construct having the formula P_1-x-P_2 has a peptide P_2 selected from one of the following groups; the group consisting of SEQ ID No.'s 967-969 and 1013-1014; the group consisting of SEQ ID No.'s 973-978 and 1016-1021; the group consisting of SEQ ID No.'s 979-980 and 1022-1023; the group consisting of SEQ ID No.'s 981-988 1024-1031; the group consisting of SEQ ID No.'s 989-990 and 1032-1033; the group consisting of SEQ ID No.'s 991-995 and 1034-1035; the group consisting of SEQ ID No.'s 996-999 and 1036-1039; the group consisting of SEQ ID No.'s 1000-1006 and 1040-1043; the group consisting of SEQ ID No.'s 1007-1011 and the group consisting of SEQ ID No.'s 1012 and 1044), or variants of any of the foregoing sequences.

In certain embodiments, the peptide construct is selected from one of the following groups: the group consisting of SEQ ID No.'s SEQ ID No.'s 291, 293, 856 and 964-965 SEQ ID No.'s 857-861; the group consisting SEQ ID No.'s 315, 317, 319, 325, 331, 339, 341, 862 and 863-870; SEQ ID No.'s 347, 828, 871-876 and 966; the group consisting SEQ ID No.'s 781, 877, 878, 879; SEQ ID No.'s 881, 883-884, 886-895, 897 and 899-900; SEQ ID No.'s 786 and 901; SEQ ID No. 902; the group consisting SEQ ID No.'s 355, 903 and 904; SEQ ID No. 804; SEQ ID No. 906; SEQ ID No. 907; the group consisting SEQ ID No.'s 908-911; the group consisting SEQ ID No.'s 912-913; the group consisting SEQ ID No. 914; SEQ ID No. 915 and SEQ ID No. 916, or variants of any of the foregoing sequences.

In certain embodiments, the peptide construct is selected from one of the following groups; the group consisting of SEQ ID No.'s 1045-1049; the group consisting of SEQ ID No.'s 1051-1056; the group consisting of SEQ ID No.'s 1057-1058; the group consisting SEQ ID No.'s 1059-1066; SEQ ID No.'s 1067-1068; the group consisting SEQ ID No.'s 1069-1073; the group consisting SEQ ID No.'s 1074-1077; the group consisting SEQ ID No.'s 1078-1084; SEQ ID No.'s 1085-1089; SEQ ID No. 1050 and SEQ ID No. 1090.

One of ordinary skill in the art will appreciate that other aspects of this invention will become apparent upon review of the following detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A-1B show response of C57BL/6 bone marrow cells to JgD, J, gD, or JH immunogen treatments. JgD, J, gD, or JH were added to the BM cell suspensions and incubated for 48 hrs. In FIG. 1A, cells from 3 mice were then stained with PE-anti-CD11c and in FIG. 1B, PE-anti-CD86, and flow cytometry was performed on cells within the suspension with light scatter parameters of monocytes. The X-mean for each evaluation indicates extent of antigen expression.

FIG. 2 shows Kaplan-Meier survival curve for mice vaccinated with either the JgD LEAPS® heteroconjugate-treated DC or untreated BM receiving lethal challenge with herpes simplex virus type 1 by zosteriform challenge.

FIG. 3 shows reduction in symptoms of mice (see FIG. 2) treated with JgD LEAPS™ heteroconjugate-treated DC as compared with: No treatment; Untreated BM; J-H; or JH LEAPS™ heteroconjugate-treated DC.

FIG. 4 represent a study where CEL-2000 treatment with 2 doses of 33 or 100 nmol was given subcutaneously on days 0 and 7 or days 0 and 14. Most regimes reduced the progression of arthritis disease to levels that were at least as good as those of mice treated with Enbrel® (every other day for the 28 days of the study). Immunization of mice with the 100 nmol dose (3x treatment) on days 0 and 7 appeared to limit the progression of disease throughout the experimental period. The CEL-2003 links the murine collagen II peptide, residues 254-273 ($CH_{254-273}$), sequence to the J ICBL. This trial suggests that the dosage and schedule of administration (time between initial and second immunization) are important parameters of CEL-2000 treatment. Use of a student "t" Test analysis of Treatment groups at day 7 days 14 and 21 to calculate the p value showed the 3x dose of CEL-2000 on day 0 and 14 followed by 3x dose on day 0 and 7 or 1x dose on day 0 and 7 is equivalent to 0 and 14 and slightly better than Enbrel® every other day for all 28 days.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides LEAPS™ peptide hetero-conjugates useful as immunomodulators for modulated the immune response to an autoimmune condition. The present invention further provides LEAPS™ peptide heteroconju-gates useful for treatment of cancers and localization of these LEAPS™ heteroconjugate-activated DCs at the site of cancer tumors and clusters of cancer cells. The DCs can be labeled to detect or visualize the site of the ongoing disease or cancer in the body of a subject. The novel heteroconju-gates disclosed herein are based upon epitope or antigen sequences associated with a protein (or peptide) for specific forms of cancer.

The LEAPS™ peptide heteroconjugates disclosed herein have a protein sequence that binds to a specific class or subclass of immune cells and a protein sequence corre-sponding with an antigen. The LEAPS™ peptide heterocon-jugates can be used to directly modulate the response of the immune system or specific immune cells to the antigen sequence. As such, the LEAPS™ peptide heteroconjugates disclosed herein can be used to direct an immune response against antigen sequences expressed by cancerous cells.

Various antigens associated with autoimmune conditions, often with defined epitopes recognized for some Human Leukocyte Antigens (HLA) genotypes, have been identified, including those associated with Insulin Dependent Diabetes Mellitus (IDDM), Rheumatoid Arthritis (RA) (e.g. collagen type II 390-402 IAGFKGEQGPKGE (SEQ ID No. 1), Systemic Lupus Erythematousis (SLE), Ankyosing Spondy-litis (AS), Pemphius Vulgaris (PV) (epidermal cell adhesion molecule desmoglein 190-204). Multiple Sclerosis (MS), Myelinproteolipid (MPL) (peptide sequence KNIVTPRT (SEQ ID No. 2), certain types of psoriasis, and uveoretintis (Hammer et al., HLA class I peptide binding specificity and autoimmunity, 1997, Adv. Immunol, 66:67 Tisch et al., Induction of Glutamic Acid Decarboxylase 65-Specific Th2 Cells and Suppression of Autoimmune Diabetes at Late Stages of Disease Is Epitope Dependent 1999, J. Immunol. 163:1178; Yoon et al., Control of Autoimmune Diabetes in NOD Mice by GAD Expression or Suppression in .beta. Cells 1999, Science 284:1183; Ruiz et al., Suppressive Immunization with DNA Encoding a Self-Peptide Prevents Autoimmune Disease: Modulation of T Cell Costimulation 1999, J. Immunol., 162:3336; Kreo et al., Identification of T Cell Determinants on Human Type II Collagen Recognized by HLA-DQ8 and HLA-DQ6 Transgenic Mice 1999, J.

Immunol, 163:1661). In other cases, peptides are known that induce in animals, a condition similar to ones found in humans, such as GDKVSFFCKNKEKKC (SEQ ID No. 3) for antiphospholipid antibodies associated with thrombosis (Gharavi et al., GDKV-Induced Antiphospholipid Antibodies Enhance Thrombosis and Activate Endothelial Cells In Vivo and In Vitro 1999, J. Immunol., 163:2922) or myelin peptides for experimental autoimmune encephalitis (EAE) as a model for MS (Ruiz et al., supra. Araga et al., A Complementary Peptide Vaccine That Induces T Cell Anergy and Prevents Experimental Allergic Neuritis in Lewis Rats 1999, J. Immunol., 163:476-482; Karin et al., Short Peptide-Based Tolerogens Without Self-Antigenic or pathogenic Activity Reverse Autoimmune Disease 1999, J. Immunol, 160:5188; Howard et al., Mechanisms of immunotherapeutic intervention by anti-CD40L (CD154) antibody in an animal model of multiple sclerosis 1999, J. Clin Invest., 103:281).

Moreover, glutamic acid decarboxylase (GAD) and specific peptides have been identified associated with IDDM (Tisch et al., supra; Yoon et al., supra). Many of these conditions are also characterized by elevated levels of one or more different cytokines and other effectors such as Tumor Necrosis Factor (TNF) (Kleinau et al., Importance of CD23 for Collagen-Induced Arthritis: Delayed Onset and Reduced Severity in CD23-Deficient Mice 1999, J. Immunol. 162: 4266; Preckel et al., Partial agonism and independent modulation of T cell receptor and CD8 in hapten-specific cytotoxic T cells 1998. Eur. J. Immunol., 28:3706; Wooley et al., Influence of a recombinant human soluble tumor necrosis factor receptor FC fusion protein on type II collagen-induced arthritis in mice 1993, J. Immunol., 151:6602) as well as auto-antibodies, including in some cases, anti-costimulator molecules, in particular, those for Cytotoxic T-lymphocyte-Associated protein 4 ((CTLA-4) (CD152)) on CD4+ cells (Matsul et al., Autoantibodies to T Cell Costimulatory Molecules in Systemic Autoimmune Diseases 1999, J. Immunol., 162:4328).

Definitions

Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the relevant art.

The articles "a" and "an" are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

The term "adjuvant" refers to substance that accelerates, prolongs or enhances antigen-specific immune responses when used in combination with vaccine antigens.

The terms "administering," "administer," "delivering," "deliver," "introducing," and "introduce" can be used interchangeably to indicate the introduction of a therapeutic or diagnostic agent into the body of a patient in need thereof to treat a disease or condition, and can further mean the introduction of any agent into the body for any purpose.

The term "antigen" refers to a substance or molecule that generates an immune response when introduced to the body or any molecule or fragment thereof now also refers to any molecule or molecular fragment that can be bound by a major histocompatibility complex (MHC).

The term "autoimmune disease" refers to a condition where a subject's own immune system directs an immune response against the subject's own cells and tissues. Autoimmune diseases include, but are not limited to, rheumatoid arthritis.

The term "blood tissue" refers to cells suspended in or in contact with plasma.

The term "bone marrow cell" refers to any cell originating from the interior of bones.

The terms "CD80," "CD86," "CD11c, "CD85" and similar terms refer to cell surface molecules present on leukocyte cells through a nomenclature protocol maintained by Human Cell Differentiation Molecules (www.hedm.org; Paris, France).

The terms "conjugate" "conjugation" and similar terms refer to two species being spatially associated with each other by covalent linkage, non-covalent binding or by a combination of covalent linkage and non-covalent binding. For example, an antibody can be conjugated to an epitope through non-covalent binding to the epitope as well as the antibody serving to conjugate the epitope (such as a cell surface marker) to a compound that is linked to the antibody.

The term "comprising" includes the recited steps, elements, structures or compositions of matter and does not exclude any un-recited elements, structures or compositions of matter.

The term "consisting of" includes and is limited to whatever follows the phrase the phrase "consisting of." Thus, the phrase indicates that the limited elements are required or mandatory and that no other elements may be present.

The phrase "consisting essentially of" includes any elements listed after the phrase and is limited to other elements that do not interfere with or contribute to the activity or action specified in the disclosure for the listed elements. Thus, the phrase indicates that the listed elements are required or mandatory but that other elements are optional and may or may not be present, depending upon whether or not they affect the activity or action of the listed elements.

A "dendritic cell" or "DC" refers to an antigen-presenting leukocyte that is found in the skin, mucosa, and lymphoid tissues, and having a capability under appropriate conditions to initiate a primary immune response by activating T cells, lymphocytes and/or secreting cytokines.

The term "diagnostic" refers to any technique for determining the presence of any particular autoimmune condition or antigen in a subject.

The term "divalent linker" refers to any moiety having a structure forming a peptide bond to a first peptide moiety and forming a second bond to a second peptide moiety.

The term "effect amount" is an amount of a therapeutic which produces a therapeutic response, including an immune response, in the subject to which the therapeutic is administered.

The term "autologous" refers to a situation where the donor and recipient of cells, fluids or other biological sample is the same person.

The term "homologous" refers to a situation where the donor are recipient of cells, fluids or other biological sample or material are not the same individual.

The term "infection" refers to the colonization in a host organism by a pathogenic agent that can include parasites, viruses, and bacteria.

An "immature dendritic cell" is a "dendritic cell" in a state characteristic of immune cells prior to contact with an antigen and having a limited present ability to active T cells, lymphocytes and/or to secrete cytokines; however, "immature dendritic cells" may acquire the ability to activate T cells, lymphocytes and secrete cytokines upon contact with an antigen.

The terms "immunomodulatory" and "immunoprotein" refer to a protein, peptide or cell having the ability to bind or interact with an immune cell to alter or to regulate one or more immune functions.

The term "Interleukin 12p70" refers to a cytokine produced by dendritic cells capable of directing the development of lymphocytes in a TH1 immune response, and possessing two peptides of approximately 40 kd and 35 kd in size.

The terms "isolated matured dendritic cells" or "isolated dendritic cells" refer to dendritic cells suspended in a liquid medium, a cell culture or a composition wherein at least 50% of the viable cells present in the liquid medium, the cell culture or the composition are dendritic cells or monocytes.

An "isotype control" is an antibody having the same serological structure and can have a fluorescent conjugate dye as an antibody conjugate having affinity for a cellular surface or cytokine marker, except the isotype control does not have affinity for the cellular surface or cytokine marker.

A "heteroconjugate" refers to a protein or peptide containing at least two amino acid sequences covalently linked to form a single molecular, wherein two sequences originate or are homologous to proteins expressed by different genes.

The term "maturation" refers to a process for generating a "matured dendritic cell."

The terms "matured dendritic cell," "maturated dendritic cell," "activated dendritic cell" or "effective dendritic cell" refer to a "dendritic cell" in a state characteristic of cells after contact with an antigen and having a present ability to initiate a primary immune response by activating T cells, lymphocytes and/or secreting cytokines.

The term "monocyte" refers to immune cells produced by bone marrow and haematopoietic stem cell having the ability to differentiate into macrophages or dendritic cells.

The term "magnetic resonance imaging" refers to any technique where information is collected from the exposure of a subject or sample to a magnetic field.

The terms "originating" and "derived" as related to peptide sequences refers to an organism or cell type that produces a protein containing

The term "paramagnetic contrast agent" refers to any agent having paramagnetic behavior in an applied magnetic field indicated by a positive magnetic susceptibility.

The terms "peptide" and "peptide construct" refer to molecule including two or more amino acid residues linked by a peptide bond. The term "peptide" indicates molecular species where only part of the molecule has peptide character and/or where two parts or the molecular species formed of peptide bonds are covalently linked by a divalent linker.

The term "phenotype" as relating to the phenotype of immune cells refers to any observable characteristic or trait of a cell such as its morphology, development, biochemical or physiological properties including the expression or presence of specific cell surface proteins or markers.

The term "prophylactic" or "prophylactically" refers to a method or use of a peptide, cells or biological matter in a manner to prevent the onset or occurrence of a disease or infection including use as a vaccine.

The term "red blood cells" refers to erythrocytes having an intact phospholipid bilayer membrane.

The term "subject" or "patient" refers to an animal, including mice and humans, to which a therapeutic agent is administered.

The term "systemic immune response" refers to an immune response where antibodies, cytokines, or immune

cells generated by the immune response are detectable throughout the circulatory and lymph systems of the body.

The term "T cell" refers to a lymphocytes having a T cell receptor protein on the surface of the cell.

The terms "treating" "treatment" as related to treating or treatment of immune cells refers to bringing an immune cell into contact with a substance or composition for a time period sufficient to cause a change in phenotype.

The term "vaccine" refers to compositions containing one or more antigens that stimulates an immune response when administered to an organism in vivo.

Structure of Immunomodulatory LEAPS™ Heteroconjugates

The peptide constructs disclosed herein are based on LEAPS™ technology and are heteroconjugates of two peptides which are linked together covalently. The peptide heteroconjugates or constructs can be synthesized artificially using solid-phase synthesis or other synthetic technique or expressed using recombinant DNA technology. The two peptides can be synthesized separately and joined covalently or can be synthesized or expressed as a single construct. A first peptide (hereinafter may be referred to as Peptide P_1) of the heteroconjugate is a portion of an immunoprotein capable of promoting binding to a class or subclass of dendritic cells (DCs) or T cells and is referred to as an immune cell binding ligand (ICBL). Without wishing to be bound by any particular theory, it is believed that Peptide P_1 has a structure for promoting interaction and/or binding with specific surface receptors present on DCs. Peptide P_1 can be a peptide sequence derived from Major Histocompatibility Complex (MHC) I or II. A more detailed discussion of the Peptide P_1 and peptide heteroconjugates involved with LEAPS™ technology can be found in U.S. Pat. No. 5,652, 342, which is incorporated herein by reference.

A second peptide (hereinafter may be referred to as Peptide P_2) is a specific antigen peptide derived from and/or associated with an autoimmune condition or derived from a cancer cell. Without being wishing to be bound by any particular theory, it is believed that the antigen Peptide P_2 being covalently linked to the ICBL Peptide P_1 allows for a more effective recognition of the antigen Peptide P_2 by the immune system and specific immune cells thus allowing for antigen-specific immunomodulation. Peptide epitopes having a limited number of amino acid residues have sufficient structure to be bound by an antibody or an MHC molecule with a high degree of specificity. However, peptide epitopes of limited size are less competent to cross-link immunoglobulins to cause lymphocyte activation and/or capable of inducing an immune reaction or immunomodulations. As such, small peptide epitopes introduced into a subject may produce a poor immune response.

In the LEAPS™ heteroconjugates disclosed herein, the antigen Peptide P_2 is covalently bound to ICBL Peptide P_1 or other immunomodulatory peptide having the capability to bind to molecules present on the surface of dendritic cells or monocytes. Once bound to the surface of a dendritic cell, the antigen Peptide P_2 can then be recognized by local T cell receptor (TCR) or Major Histocompatibility Complex (MHC) molecules to trigger a corresponding immune response and/or immunomodulation through an immune recognition of the antigen Peptide P_2. In certain embodiments, DCs are treated or contracted with a LEAPS™ heteroconjugate ex vivo away from a subject's body and then administered to the subject. Through such a mechanism, the immune system of a subject to whom the LEAPS™ heteroconjugate-activated DCs are administered can be modulated to have a modulated response to a source

of antigen with the body involved with the autoimmune condition. In certain other embodiments, the LEAPS™ heteroconjugate-activated DCs when administered to a subject locate to the site of a source of the antigen, whether such antigen source is a self-antigen involved in an autoimmune condition. The LEAPS™ heteroconjugate-activated DCs can be associated or conjugated with a radio label, dye, therapeutic compound or source of ionizing radiation to assist with the detection or imaging of the antigen source or to deliver the therapeutic compound or ionizing radiation to the site of the antigen source.

A further aspect of the LEAPS™ heteroconjugates disclosed herein is that the extent of pro-inflammatory or inflammatory cytokines produced during the immune response to the peptide constructs is reduced relative to levels typically associated with larger antigen proteins containing many different epitope sequences. Further, a Th1 type of immune response or a Th2 type of immune response may be promoted based upon the identity of the ICBL Peptide P_1 conjugated with the antigen Peptide P_2.

A further aspect of the LEAPS™ heteroconjugates disclosed herein is that the heteroconjugates can be treated or contacted with dendritic cells isolated from a subject or donor under conditions where the dendritic cells differentiate into more matured immune cells capable of directing immunity toward the antigen peptide sequence contained within the LEAPS™ heteroconjugates. The matured DCs can modulate immune response to sources of the antigen within the body of a subject to whom the matured DCs are administered.

The LEAPS™ heteroconjugates disclosed herein can assist in generating an active immune response to an antigen derived from a cancer cell. However, LEAPS™ heteroconjugates can also operate to downwards regulate the immune response to self antigens in individuals with an active autoimmune disease. As the Example below demonstrate, the LEAPS™ heteroconjugates disclosed herein have the capability to modulate an undesirable autoimmune response and cause a reduction in symptoms. The exact mechanism for immune modulation and/or a decrease in immune response in an antigen-specific matter is not fully known, and Applicants do not wish be bound by any particular theory regarding the mechanism of operation of the LEAPS™ heteroconjugates disclosed herein. In a study of the affect of LEAPS™ heteroconjugates in experimental autoimmune myocarditis (EAM), immunization of A/J mice with a LEAPS™ heteroconjugate having the pathogenic My-1 peptide from murine cardiac myosin linked to "J" peptide (described below) conferred both protection and treatment against EAM. These findings were for a L.E.A.P.S. vaccine protecting against EAM, a condition induced in A/J mice with the My-1 peptide from murine cardiac myosin. While the J-My-1 vaccine was not evaluated with other models, this condition can be induced by coxsackie virus B3 infection as well as immunization with murine cardiac myosin (MCM) 1. Therapies for EAM induced by My-1 such as monoclonal antibodies (for TNF-.alpha or IL-1, beta.), anti-complement receptor, cobra venom or recombinant proteins such as IFN-gamma are effective only if given in the first week, during the induction phase but are ineffective when given by day 10 or later. (Cihakova D. J G. Barin, M Kimura, G C Baldeviano, M V Talor, D H Zimmerman, E Taylor, N R Rose, 2008 Conjugated Peptide Ligand is Able to Prevent and Treat Experimental Autoimmune Myocarditis, is a Strong Stimulator of Cell and Humoral Immunity. *Int Immunopharmacol* 8:625-633 (which is incorporated herein by reference).

One possible conclusion is that the LEAPS™ heteroconjugate vaccine J-My1 was antigen specific (for My-1), did not induce a general anergy as no effect for the anti PPD response, had little effect on antibody to My-1, reduced proliferative responses to My-1 and did this without acting as a general mitogen or polyclonal activator. Expanded numbers of activated CD69+, CD44+, CD4+, and CD8+ cells, as well as increased CD11c+ DCs were observed in the spleens. No differences in CD4+, CD25+, and FoxP3+ Treg cell numbers were detected in the spleen or the target heart organ. Examination of the chemokine and cytokine response with the Quantikine ELISA kits for IFN-gamma, TGF-13, TNF-alpha, IL-1-alpha, IL4, IL10, IL2. Histamine, IP-10, MIP-1-alpha of sera and spleens were unremarkable, however cardiac tissue showed a significant decrease in MIP-1-alpha and IP10. This is in contrast to the elevated levels of these molecules in another EAM model and the ability of monoclonal antibody ablation to MIP-1-alpha or MCP-1 to reduce disease severity. Although IL-17 may be involved, it was not studied as reagents were not available.

Regardless of the theoretical mechanism for the action of LEAPS™ heteroconjugate vaccines in modulating immune response, the disclosure shows how to make and use effective LEAPS™ heteroconjugates.

LEAPS™ Heteroconjugates

Specifically, the novel peptide heteroconjugates of this invention include peptide constructs of the following Formulae (I) and (II):

$$P_1\text{-x-}P_2 \tag{I}$$

$$P_2\text{-x-}P_1 \tag{II}$$

where peptide P_2 is an antigen peptide associated with an autoimmune disease condition or associated with a cancer cell. It is believed that the antigen peptide P_2 binds to an antigen receptor on a set or subset of dendritic cells or T cell. P_1 is an immune response modifying peptide, which will cause a directed immune response by said set or subset of DCs or T cells to which the peptide P_1 is bound and modulates an immune response focused on IL-12 without or with low levels of pro-inflammatory or inflammatory cytokines (Patricia R Taylor; Christopher A Paustian, Gary K Koski, Daniel H Zimmerman, K S Rosenthal. Maturation of dendritic cell precursors into IL12 producing DCs by J-LEAPS. *Cellular Immunology*, 2010, 262:1-5; Taylor P R, G K Koski, C C Paustian, P A Cohen, F B-G Moore, D H Zimmerman, K S Rosenthal, J-L.E.A.P.S.™ Vaccines Initiate Murine TH1 Responses By Activating Dendritic Cells, *Vaccine* 2010: 28:5533-4, both of which are incorporated herein by reference). As shown in Formulae (I) and (II), the Peptide P_1 can be N-terminal or C-terminal to the Peptide P_2.

In certain embodiments, the Peptide P_1 contains an ICBL termed "J" or "Peptide J." Peptide J is derived from amino acids 38-50 from the β-2-microglobulin chain of the MHC 1 molecule (DLLKNGERIEKVE) (SEQ ID No. 49). ICBL Peptide J is believed, but is not limited to, promoting Th1-type immune responses to the coupled antigen P_2 peptide.

In certain embodiments, the Peptide P_1 of the peptide constructs contains an ICBL termed "CEL-1000" (DGQEEKAGVVSTGLI) (SEQ ID No. 48). The CEL-1000 peptide is derived from the β-chain of MHC II (MHC II β134-148) and binds to murine as well as human CD4+ cells. The chemical structure of conjugated peptides containing CEL-1000 can have an amidated carboxyl terminal, (amino)-DGQEEKAGVVSTGLI-(amide). CEL-1000 can be prepared by F-MOC chemistry and purified by Reverse

Phase (RP)-HPLC, analyzed by another RP-PLC system, ion exchange chromatography (IEC)-HPLC as well as mass spectroscopy. Based on site directed mutagenesis studies of MHC II β-chain and/or peptide competition studies, peptides such as CEL-1000, were shown to bind to CD4, a T cell co-stimulator molecule (Cammarota et al., Identification of a CD4 binding site on the beta 2 domain of HLA-DR molecules. Nature, 1992: 356:799-801) and cell surface protein on some Dendritic Cell (DCs) (Konig, et. al., MHC class II interaction with CD4 medicated by a region analogous to the MHC class I binding site for CD8, Nature, 1992: 356:796-798; Shen X. and Konig R., "Regulation of T cell immunity and tolerance in vivo by CD4", Int. Immunol., 1998 10:247-57; Shen X. et al., Peptides corresponding to CD4-interacting regions of murine MHC class II molecules modulate immune responses of CD4+ T lymphocytes in vitro and in vivo, J. Immunol., 1996; 157:87-100, all of which are incorporated herein by reference).

In certain embodiments, the Peptide P_1 contains an ICBL termed "G" or "peptide G." Peptide G has the sequence NGQEEKAGVVSTGLI (SEQ ID No. 15) derived from the MHC-II beta 2 chain (Zimmerman et al., A new approach to T cell activation: natural and synthetic conjugates capable of activating T cells, 1996, Vacc. Res., 1996: 5:91, 5:102: Rosenthal et al., Immunization with a LEAPS™ heteroconjugate containing a CTL epitope and a peptide from beta-2-microglobulin elicits a protective and DTH response to herpes simplex virus type 1, 1999, Vaccine, 1999: 17(6): 535-542, both of which are incorporated herein by reference). In another embodiment, the Peptide P_1 contains Hu IL-10 and has the sequence DNQLLETCKQDRLRN-RRGNGSSTHFEGNLPC (SEQ ID No. 27) (Gesser et al., Identification of functional domains on human interleukin 10 1997, Proc. Nat. Acad. Sci. 94:14620).

In certain embodiments, the Peptide P_1 contains an ICBL termed "IL-1β" or "Peptide IL-1β." Peptide IL-1β has the sequence VQGEESNDK (SEQ ID No. 13) derived from the human interleukin-1β chain (e.g., Bajpai et al., Immunomodulating activity of analogs of noninflammatory fragment 163-171 of human interleukin-1beta 1998 Immunopharmacology, 38:237, incorporated herein by reference).

In certain addition embodiments, the Peptide P_1 contains ICAM-1 LFA-3 (aa26-42). VLWKKQKDKVAELENSE (SEQ ID No. 4); a TNF-α ligand portion such as amino acids 70-80 PSTHVLITHTI (SEQ ID No. 5); or the peptide represented by peptide represented by DFLPHYKNT-SLGHRP (SEQ ID No. 6).

Epitope sequences that can serve as the antigen Peptide P_2 and conjugated with the ICBL Peptide P_1 to form a LEAPS™ heteroconjugate will now be described. The antigen Peptide P_2 can be selected from protein sequences associated with different types of autoimmune conditions, including Alzheimer's dementia (immune response to ameloid β protein implicated), myocarditis, diabetes mellitus, rheumatoid arthritis, pemphigus vulgaris, multiple sclerosis, uveoretinitis, thrombosis, myastmenia gravis, psoriasis, pernicious anemia, autoimmune hepatitis, systemic lupus erythematosus, rheumatic fever, Graves disease, systemic sclerosis, and Goodpature's syndrome. While Alzheimer's dementia may not generally be considered to be a disorder of the immune system, plaques formed by Alzheimer's can be treated in a similar manner as autoimmune diseases. As such, Alzheimer's disease is considered to be within the scope of autoimmune diseases treatable through the use of LEAPS™ heteroconjugates as disclosed herein.

In other embodiments, epitope sequences that can serve as the antigen P_2 peptide and conjugated with the ICBL Peptide P_1 to form a LEAPS™ heteroconjugate can be selected from protein sequences associated with different types of cancer including liver, colon, breast, cervical, melanoma, prostate, ovarian, colorectal, gastric, lung and cervical cancers. Protein sequences and antigens associated with cancer ultimately derive from the host's own genome. As such, antigens associated with cancer can have a weak immune response due to suppression of the recognition of self-antigens by a host. However, some cancer cells express proteins, some of which are present on the surface of cancer cells that contain mutations compared to surrounding non-cancerous cells. However, even the presence of mutated proteins in cancers often does not engender a strong host immune response since such mutated protein sequences are often not processed by antigen presenting cells (APCs), which is a typical first step in directing an immune response against a specific antigenic peptide sequence.

LEAPS™ heteroconjugates having immunomodulatory effects on specific autoimmune conditions or having immunomodulatory effects toward cancer are contemplated containing any combination of sequences selected from embodiments of Peptide P_1 ICBLs, described above, and Peptide P_2 presented on Table 1 for autoimmune diseases or Table 2 for cancer having the structure of one of Formulae (I) and (II), as described above. In Formulae (I) and (II), -x- represents a covalent bond or a divalent peptide linking group providing a covalent linkage between Peptide P_1 and Peptide P_2. In certain embodiments, -x- is a divalent peptide linking group having one or more glycine residues, such as the divalent linking group -GGG- or -GG-. In order to avoid synthesis of peptides having four glycine residues in a row, which may be hard to synthesize, a linking group of -GG- can be used. Divalent linkers containing the residue serine can also be present in the divalent linking group, -x-. For example, divalent linking group can be -GGSG- (SEQ ID No. 1092), -GGGS- (SEQ ID No. 7) or -GGGGS- (SEQ ID No. 1093).

In certain embodiments, the divalent linking group is not limited to any particular identity so long as the linking group -x- serves to covalently attach the Peptide P_1 and Peptide P_2 as shown in Formulae (I) and (II). The linking group -x- can contain one or more amino acid residues or a bifunctional chemical linking group, such as, for example, N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), m-maleimido-benzoyl-N-hydroxy-succinide ester (MBS), or 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC). In certain embodiments, the linking group -x- can be a direct peptide or other covalent bond directly coupling Peptide P_1 and Peptide P_2. In certain embodiments where the linking group -x- contains amino acid residue, the linking group -x- can contain from 1 to about 5 amino acid residues or from 1 to about 3 amino residues. In certain embodiments, the linking group -x- can be cleavable or non-cleavable under physiological conditions.

The LEAPS™ heteroconjugates of Formulae (I) and (II) can be modified including modifications to the N- or C-terminal or the heteroconjugates. The LEAPS™ heteroconjugates described by Formulae (I) and (II) contain a sequence of amino acid residues consistent with the described Peptide P_1 and Peptide P_2. However, the N- or C-terminal of the described LEAPS™ heteroconjugates can be modified by any one or more of amidation or acylation, including myristoylation.

In certain embodiments, Peptides P_1 and P_2 and peptide heteroconjugates including peptides P_1 and P_2 include variants of any sequence presented herein in SEQ ID No.'s

1-1090. A variant is herein defined as a sequence wherein 1, 2, 3, 4 or 5 amino acid residues of any sequence disclosed herein are replaced with a different amino acid residue without affecting the ability of the LEAPS™ heteroconjugates to stimulate an immune response. In certain embodiment, variants have amino acid residues substituted in a conserved manner. In certain other embodiments, variants to SEQ ID No.'s 1-1090 have amino acid residues substituted in a non-conserved manner. Variants to SEQ ID No.'s 1-1090 include amino acid sequences where 1, 2, 3, 4 or 5 amino acid residues are deleted from the sequences and/or 1, 2, 3, 4 or 5 amino acid residues are added to the sequences. Variants include embodiments where combinations of conserved or non-conserved substitutions, additions and/or deletions are made to a sequence.

A conserved substitution is a substitution where an amino acid residue is replaced with another amino acid residue having similar charge, polarity, hydrophobicity, chemical functionality, size and/or shape. Substitution of an amino acid residue in any of the following groups with an amino acid residue from the same group is considered to be a conserved substitution: 1) Ala and Gly; 2) Asp and Glu; 3) Ile, Leu, Val and Ala; 4) Lys, Arg and His; 5) Cys and Ser; 6) Phe, Trp and Tyr; 7) Phe and Pro; 8) Met and Nle (norleucine); 9) Asn and Gln; and 10) Thr and Ser.

Table 1 shows exemplary antigens that can be employed as Peptide P_2 in certain embodiments. LEAPS™ heteroconjugates consistent with Formulae (I) and (II) can be formed by combining any permutation of ICBL peptide P_1 (e.g., CEL-1000, Peptide J and/or Peptide G, etc.) with an antigen (Peptides P_2) as presented in Table 1. Table 1 lists antigen sequences grouped by the condition or disease that such sequences are associated with. Specifically, the first column

of Table 1 list the SEQ ID No. for the sequence presented in each row. The second column lists the disease, such as an autoimmune condition, for which the sequences presented in each row relate. The third column specifies the protein from which individual amino acid sequences are derived. The fourth column gives the abbreviation for which the sequence presented in each row can be referred to. For example, AB1-42 stands for ameloid β protein, residues 1-42, whereas J-AB1-42 indicates a LEAPS™ heteroconjugate having the ICBL Peptide J linked to ameloid β protein, residues 1-42. Also provided on Table 1 are example LEAPS™ heteroconjugates, where Peptide P_1 is Peptide J (SEQ ID No. 49) combined with an antigen Peptide P_2. The fifth Column specifies the core epitope sequence, if any, for the protein described in each row, and the sixth column specifies an extended epitope sequence associated with the protein described in each row. The seventh column indicates the range of amino acids from the described protein corresponding to the epitope sequence. The eighth column specifies an exemplary LEAPS™ heteroconjugate where the ICBL Peptide J (SEQ ID No. 49) is linked to one of the described antigens through a triglycine linker. Those skilled in the art will recognize that other LEAPS™ heteroconjugate constructs can be formed substituting for Peptide P_1 and Peptide P_2, where the examples on Table 1 are merely illustrative and are not limiting. The ninth column lists any know references describing the extended or core epitope sequences, if known. References are specified by a number corresponding to the list of references found at the end of this disclosure.

In addition to the antigen sequences disclosed in Table 1, U.S. Paten Publication 2006/0257420 A1 and U.S. Patent Publication U.S. Patent Publication 2011/0098444 A1 are expressly incorporated herein by reference.

TABLE 1

Autoimmune related antigens and example LEAPS™ heteroconjugates

Seq ID No.	Disease	Protein Candidates	Abbreviation	Core epitope	Extended region	Amino acid position	J LEAPS conjugate	Ref.
51	Alzheimers dementia ameloid b protein		Ab1-42	NA	DAEFRHDSGYEVHHQKLVFFAED VGSNKGAIIGLMVGGVV	1-42	NA	3
291			J-Ab1-42	NA	NA	NA	DLLKNGERIEKVEGGGDAEFRHDSGYEV HHQKLVFFAEDVGSNKGAIIGLMVGGVV	66
53	Alzheimers dementia ameloid b protein		Ab1-28	NA	DAEFRHDSGYEVHHQKLVFFAEN VGSNK	1-28	NA	3, 4
293			J-Ab1-28	NA	NA	NA	DLLKNGERIEKVEGGGDAEFRHDSGYEV HHQKLVFFAENVGSNK	
55	Alzheimers dementia ameloid b protein		Ab1-30	NA	DAEFRHDSGYEVHHQKLVFFAEN VGSKAI	1-30	NA	3, 4
856			J-Ab1-30	NA	NA	NA	DLLKNGERIEKVEGGGDAEFRHDSGYEV HHQKLVFFAENVGSKAI	
960	Alzheimers dementia ameloid b protein		Ab1-15		DAEFRHDSGYEVHHQ	1-15	NA	75, 76, 77
964			J-Ab1-15				DLLKNGERIEKVEGGGDAEFRHDSGYEV HHQ	
961	Alzheimers dementia ameloid b protein		Ab12-33		KLVFFAEDVGSNKGAIIG	16-33	NA	77, 78, 79
965			J-Ab33				DLLKNGERIEKVEGGGKLVFFAEDVGSN KGAIIG	
962	Rheumatoid ArthritisType II Cplagen		CII-354	GARGLTGRPGDA	NA	354-365	NA	80
963			CII-350		PGLPGARGLTGRPGDAGPQG	350-369	NA	81
966			J-CII-350				DLLKNGERIEKVEGGGPGLPGARGLTGR PGDAGPQG	
749	Myocarditis Myosin		My4	NA	KRKLEGDLKLTQESIMDLENDKQQL	800-824	NA	5
857			J-My4	NA	NA	NA	DLLKNGERIEKVEGGGKRKLEGDLKLTQ ESIMDLENDKQQL	40
72	Myocarditis cardiac antigen trponin I		CATI	NITEIADLTQK	NA	121-131	NA	6
917			CATIext	NA	AKVTKNITEIEADLTQKIFDLR	116-136	NA	41
858			J-CATIext	NA	NA	NA	DLLKNGERIEKVEGGGAKVTKNITEIEA DLTQKIFDLR	

TABLE 1 -continued

Autoimmune related antigens and example LEAPS ™ heteroconjugates

Seq ID No.	Disease	Protein Candidates	Abbreviation	Core epitope	Extended region	Amino acid position	J LEAPS conjugate	Ref.
751	Myocarditis		BCKD-E2 116-134	NA	NA / VRRALMENNIKLSEVVGSG	116-134	NA	7, 67
859			J-BKD116	NA	NA	NA	DLLKNGERIEKVEGGGVRRALMENNIKL SEVVGSG	
753	Myocarditis		LMM1.1	NA	NA / KEALISSLTRGKLTYTQQ	NA	NA	8
860			J-LMM1	NA	NA	NA	DLLKNGERIEKVEGGGKEALISSLTRGK LTYTQQ	40
755	Myocarditis		LMM33	NA	NA / SERVQLLHSQNTSLINQK	NA	NA	8
861			J-LMM33	NA	NA	NA	DLLKNGERIEKVEGGGSERVQLLHSQNT SLINQK	40
957	Diabetes mellitus	Insulin Growth Factor Receptor I	IGF1R	SFGVVLWEI	NA	1196-1204	NA	37, 29
958			IGF1Rext	NA	YSDVWSFGVVLWEIATLAE	1191-	NA	42
862			J-IGF1Rext	NA	NA	NA	DLLKNGERIEKVEGGGYSDVWSFGVVLW EIATLAE	
84	Diabetes	Bovine serum albumin	ABBOS 152	NA	NA / FKADEKKFWGKYLYE	152	NA	13, 68
325			J-ABBOS152	NA	NA	NA	DLLKNGERIEKVEGGGFKADEKKFWGKY LYE	
86	Diabetes	Insulin beta chain	Insβ 9	NA	NA / SHLVEALYLVCGERG	9	NA	14, 69
863			J-Insβ9	NA	NA	NA	DLLKNGERIEKVEGGGSHLVEALYLVCG ERG	
88	Diabetes	Heat shock protein HSP277		NA	NA / VLGGGCALLRCIPALDSLTPANED	277	NA	15, 70
864			J-HSP277	NA	NA	NA	DLLKNGERIEKVEGGGVLGGGCALLRCI PALDSLTPANED	
90	Diabetes		RVEp151	NA	NA / EACVTSWLWSGEGAVFYRVDLH FINLGT	151	NA	16, 71
331			J-RVEp151	NA	NA	NA	DLLKNGERIEKVEGGGEACVTSWLWSGE GAVFYRVDLHFINLGT	
92	Diabetes		RVEp87	NA	NA / MDFWCVEHDRPPPATPTSLTT	87	NA	16, 71
341			J-RVEp87	NA	NA	NA	DLLKNGERIEKVEGGGMDFWCVEHDRPP PATPTSLTT	

TABLE 1 -continued

Autoimmune related antigens and example LEAPS™ heteroconjugates

Seq ID No.	Disease	Protein Candidates	Abbreviation	Core epitope	Extended region	Amino acid position	J LEAPS conjugate	Ref.
74	Diabetes	Glutamic acid decarboxylase p65	GAD65-247	NA	NA	247-265	NA	9, 43
317			J-GAD65-247	NA	NMYAMMIARFKMFPEVKEKGMAALPRLIAFTSEHSHFSLK	NA	DLLKNGERIEKVEGGGNMYAMMIARFKMFPEVKEKGMAALPRLIAFTSEHSHFSLK	
76	Diabetes	Glutamic acid decarboxylase p65	GAD65-253	NA	NA	253	NA	43
317			J-GAD65-253	NA	LARPKMFPEVKEKGMAALPRIAFTSEHSHFSLK	NA	DLLKNGERIEKVEGGGLARPKMFPEVKEKGMAALPRIAFTSEHSHFSLK	
78	Diabetes	Glutamic acid decarboxylase p65	GAD65-524	NA	NA	524	NA	10, 43
319			J-GAD65-524	NA	SRLSKVAPVIKARMMEYGTT	NA	DLLKNGERIEKVEGGGSRLSKVAPVIKARMMEYG	
80	Diabetes	Glutamic acid decarboxylase p65	GAD65-506	NA	NA	506	NA	11, 43
865			J-GAD65-506	NA	IPPSLRYLEDEERMSRLSK	NA	DLLKNGERIEKVEGGGIPPSLRYLEDEERMSRLSK	
82	Diabetes	Glutamic acid decarboxylase p65	GAD65-201	NA	NA	201	NA	12, 43
866			J-GAD65-201	NA	NTNMFTYEIAPVFVLLEYVT	NA	DLLKNGERIEKVEGGGNTNMFTYEIAPVFVLLEYVT	
98	Diabetes	Glutamic acid decarboxylase p65	GAD274	NA	NA	274	NA	17, 43
339			J-GAD274	NA	IAFTSEHSHFSLK	NA	DLLKNGERIEKVEGGGIAFTSEHSHFSLK	
103	Diabetes	Glutamic acid decarboxylase p65	GAD122	DRSTKVIDFH	NA	127-136	NA	17
918				NA	NA	122-141	NA	43
867			J-GAD122	NA	VVKSFDRSTKVIDFHYPNEL	NA	DLLKNGERIEKVEGGGVVKSFDRSTKVIDFHYPNEL	
449	Diabetes	Glutamic acid decarboxylase p65	GAD654	NA	NA	654-674	NA	17, 43
868			J-GAD654	NA	VSSVSSQFSDAAQASPSSHSS	NA	DLLKNGERIEKVEGGGVSSVSSQFSDAAQASPSSHSS	
452	Diabetes	Glutamic acid decarboxylase p65	GAD797	NA	NA	797-717	NA	17, 43
869			J-GAD797	NA	MVWESGCTVIVMLTPLVEDGV	NA	DLLKNGERIEKVEGGGMVWESGCTVIVMLTPLVEDGV	

TABLE 1 -continued

Autoimmune related antigens and example LEAPS™ heteroconjugates

Seq ID No.	Disease	Protein Candidates	Abbreviation	Core epitope	Extended region	Amino acid position	J LEAPS conjugate	Ref.
469	Diabetes	Glutamic acid decarboxylase p65	GAD854	NA	NA	854-872	NA	17, 43
870			J-GAD854	NA	FYLKNVQTQETRTLTQFHF	NA	DLLKNGERIEKVEGGGFYLKNVQTQETRTLTQFHF	72
812	Rheumatoid arthritis	Collagen Type II Human collagen Type II	CEL-2000	NA	TGGKPGIAGFKGEQGPKGEP	254-273	NA	72
828				NA	NA	NA	DLLKNGERIEKVEGGGTGGKPGIAGFKGEQGPKGEP	
1	Rheumatoid Arthritis	Collagen Type II	C-IIx	IAGFKGEQGPKGE	NA	399-402	NA	1
347			J-CIIx	NA	NA	NA	DLLKNGERIEKVEGGGIAGFKGEQGPKGE	72
474	Rheumatoid arthritis	Human collagen Type II	G54	NA	DGEAGKPGAGERGPPGPQG	54-73	NA	18, 72
871			J-G54	NA	NA	NA	DLLKNGERIEKVEGGGDGEAGKPGKAGERGPPGPQG	
477	Rheumatoid arthritis	Human collagen Type II	K94	NA	GLDGAKGEAGAPGVKGESGS	94-113	NA	18, 72
872			J-K94	NA	NA	NA	DLLKNGERIEKVEGGGGLDGAKGEAGAPGVKGESGS	
482	Rheumatoid arthritis	Human collagen Type II	P544	NA	ERGAAGIAGDKGDRGDVGEK	544-573	NA	18, 72
873			J-P544	NA	NA	NA	DLLKNGERIEKVEGGGERGAAGIAGDKGDRGDVGEK	
487	Rheumatoid arthritis	Osteopontin	OPN143	SLAYGLR	DGRGDSLAYGLRSKSKK	148-154 143-159	NA	19, 44
874			J-OPN143	NA	NA	NA	DLLKNGERIEKVEGGGDGRGDSLAYGLRSKSKK	
490	Rheumatoid arthritis	naJP1	DNAJ1	NA	QKRAAYKQYGHAAFE	NA	NA	20, 45
875			J-DNAJ1	NA	NA	NA	DLLKNGERIEKVEGGGQKRAAYKQYGHAAFE	
493	Rheumatoid arthritis	dnaJPV	DNAJV	NA	ERAAYDQYGHAAFE	461	NA	20, 73
876			J-DNAJV	NA	NA	NA	DLLKNGERIEKVEGGGERAAYDQYGHAAFE	

TABLE 1 -continued

Autoimmune related antigens and example LEAPS ™ heteroconjugates

Seq ID No.	Disease	Protein Candidates	Abbreviation	Core epitope	Extended region	Amino acid position	J LEAPS conjugate	Ref.
919	Pemphigus vulgaris	Cadherin	Cad1	GGGTGGGGG	NA	394-403	NA	37.12
920			Cad1ext	NA	CRVLGGGTGGGGGLGGPG	389-408	NA	46
877			J-Cad1ext	NA	NA	NA	DLLKNGERIEKVEGGGCRVLGGGTGGG GGLGGPG	
921	Pemphigus vulgaris	Cadherin	Cad2	AVAAVAAAG	NA	19-27	NA	37.12
922			Cad2ext	NA	CLGLLAVAVAAGANPAQ	14-31	NA	47
878			J-Cad1ext	NA	NA	NA	DLLKNGERIEKVEGGGCLGLLAVAAVA AAGANPAQ	
496	Pemphigus vulgaris	epidermal cell adhesion molecule desmoglein	DG342-358	NA	NA	342-358	NA	21
782				NA	SVKLSIAVKNKAEFHQS	NA	DLLKNGERIEKVEGGGSVKLSIAVKNK AEFHQS	48
499	Pemphigus vulgaris	epidermal cell adhesion molecule desmoglein	DG376-392	NA	NA	376-392	NA	21
879			J-DG376	NA	NVREGIAFRPASKFTV	NA	NA	48
					NA		DLLKNGERIEKVEGGGNVREGIAFRPA SKFTV	
2	Multiple Sclerosis	Mylein basis protein	MBP	KNIVTPRT	NA	118-125	NA	49
959			MBPext	NA	VVHFFKNIVTPRTPPPSQ	113-130	NA	
881			J-MBPext	NA	NA	NA	DLLKNGERIEKVEGGGVVHFFKNIVTP RTPPPSQ	
524	Multiple Sclerosis	Proteolipoprotien	PLP	HSLGKWLGHPDKF	NA	139-151	NA	24
924			PLPext	NA	LERVCHSLGKWLGHPDKFVGITY	134-156	NA	50
883			J-PLPext	NA	NA	NA	DLLKNGERIEKVEGGGLERVCHSLGKW LGHPDKFVGITY	
527	Multiple Sclerosis	Peripheral Nerve protein P2	P2	TEISFKLGQEF	NA	61-71	NA	25
925			P256	NA	STFKNTEISFKLGQEFEETTA	56-76	NA	51
884			J-P256	NA	NA	NA	DLLKNGERIEKVEGGGSTFKNTEISFK LGQEFEETTA	
549	Multiple Sclerosis	Proteolipoprotein	PLP175	NA	NA	175-192	NA	23, 50
886			J-PLP175	NA	IYFNTWTTCQSIAFPSKT	NA	DLLKNGERIEKVEGGGIYFNTWTTCQS IAFPSKT	
552	Multiple Sclerosis	myelin-associated oligodendrocytic	MOGP15	NA	NA	15-36	NA	26, 52
887			J-MOGP15	NA	QKFSEHFSIHCCPPFTFLNSSKR	NA	DLLKNGERIEKVEGGGQKFSEHFSIHC CPPFTFLNSSKR	

TABLE 1 -continued

Autoimmune related antigens and example LEAPS ™ heteroconjugates

| Seq ID No.	Disease	Protein Candidates	Abbreviation	Core epitope	Extended region	Amino acid position	J LEAPS conjugate	Ref.
555 888	Multiple Sclerosis	myelin-associated oligodendrocytic	MOGP1-20 J-MOGP1-20	NA NA	NA GQPRVIGPRHPIRALVGDEV NA	1-20 NA	NA NA DLLKNGERIEKVEGGGGQPRVIGPRHPIRALVGDEV	26, 52
572 889	Multiple Sclerosis	myelin-associated oligodendrocytic	MOGP31 J-MOGP31	NA NA	NA NATGMEVGWYRPPFSRVVHL NA	31-50 NA	NA NA DLLKNGERIEKVEGGGNATGMEVGWYRPPFSRVVHL	26 52
588 890	Multiple Sclerosis	myelin-associated oligodendrocytic	MOGP91 J-MOGP91	NA NA	NA SDEGGFTCFFRDHSYQEEAA NA	91-110 NA	NA NA DLLKNGERIEKVEGGGSDEGGFTCFFRDHSYQEEAA	26 52
581 891	Multiple Sclerosis	Proteolipoprotein	MOGP61 J-MOGP61	NA NA	NA QAPETRGRTELLKDAIGEGK NA	61-80 NA	NA NA DLLKNGERIEKVEGGGQAPETRGRTELLKDAIGEGK	26, 52
597 892	Multiple Sclerosis	Mylein basis protein	MBP85 J-MBP85	NA NA	NA ENPVVHFFKNIVTPR NA	85-99 NA	NA NA DLLKNGERIEKVEGGGENPVVHFFKNIVTPR	27, 49
606 893	Multiple Sclerosis	Proteolipoprotein	PLP184 J-PLP184	NA NA	NA QSLAFPSKTSASIGSL NA	184-199 NA	NA NA DLLKNGERIEKVEGGGQSLAFPSKTSASIGSL	28, 50
609 894	Multiple Sclerosis	Proteolipoprotein	PLP190 J-PLP190	NA NA	NA SKTSASIGSKCADARMYGVL NA	190-209 NA	NA NA DLLKNGERIEKVEGGGSKTSASIGSKCADARMYGVL	28, 50
612 950 895	Multiple Sclerosis	myelin-associated oligodendrocytic	MOG97 J-MOG97	FFRDHSYQE NA NA	NA GGFTCFFRDHSYQEEAAME NA	97-108 92-113 NA	NA NA DLLKNGERIEKVEGGGGFTCFFRDHSYQEEAAME	29 52
623 897	Multiple Sclerosis	Proteolipoprotein	PLP40 J-PLP40	NA NA	NA TGTEKLIETYFSKNYQDYEYL NA	40-60 NA	NA NA DLLKNGERIEKVEGGGTGTEKLIETYFSKNYQDYEYL	50

TABLE 1 -continued

Autoimmune related antigens and example LEAPS™ heteroconjugates

Seq ID No.	Disease	Protein Candidates	Abbreviation	Core epitope	Extended region	Amino acid position	J LEAPS conjugate	Ref.
636	Multiple Sclerosis	myelin-associated oligodendrocytic	MOG37	NA	NA	37-52	NA	31,
899			J-MOG37	NA	VGWYRPPFSRVVHLYR NA	NA	NA DLLKNGERIEKVEGGGVGWYRPPFSRV VHLYR	52
641	Multiple Sclerosis	myelin-associated oligodendrocytic	MOG145	NA	NA	145-160	NA	31
900			J-MOG145	NA	VFLCLQYRLRGKLRAE NA	NA	NA DLLKNGERIEKVEGGGVFLCLQYRLRG KLRAE	52
653	Uveoretinitis	interphotoreceptor retinoid binding protein	IRBP	NA	NA	1202-1215	NA	32
901			J-IRBP	NA	DGSSWEGVGVPDV NA	NA	NA DLLKNGERIEKVEGGGDGSSWEGVGVV PDV	53
664	Uveoretinitis	interphotoreceptor retinoid binding protein	IRBP1	NA	NA	1-20	NA	33,
786			J-IRBP1	NA	GPTHLFQPSLVLDMAKVLLD NA	NA	NA DLLKNGERIEKVEGGGPTHLFQPSLV LDMAKVLLD	53
927	Thrombosis	Beta-2-Glycoprotein 1	GP1 GP1ext	GDKVSFFCKNKEKKCNA NA	NGMLHGDKVSFFCKNKEKKCSYTED NA	274-288 269-293	NA NA	2
902			J-GP1ext	NA	NA	NA	DLLKNGERIEKVEGGGNGMLHGDKVSF FCKNKEKKCSYTED	54
19	Myasthenia Gravis	acetyl cholinereceptor Receptor	AchR 129	NA	NA	129-145	NA	34,
355			J-ArchR129	NA	EIIVTHPFDEQNCSMK NA	NA	NA DLLKNGERIEKVEGGGEIITVTHPFPDE QNCSMK	55
737	Myasthenia Gravis	acetyl cholinereceptor	AchR 259	VIVELIPSTSSAV NA	NA	259-271 254-276	NA	34, 35
951			J-ArchR259	NA	TVFLLVIVELIPSTSSSAVPLIGK NA	NA	NA DLLKNGERIEKVEGGGTVFLLIVELI PSTSSAVPLIGK	55
903				NA				
738	Myasthenia Gravis	acetyl cholinereceptor Receptor	AchR 195	NA	NA	195-212	NA	35,
904			J-ArchR195	NA	DTPYLDITYHFVMQRLPL NA	NA	NA DLLKNGERIEKVEGGGDTPYLDITYHF VMQRLPL	55
774	Psorasis	Pso P27		NA	NA	18 Jan.	NA	36
804				NA	SVDRSGNVHHQFQKLTLE NA	NA	NA DLLKNGERIEKVEGGGSVDRSGNVHHQ FQKLTLE	74

TABLE 1 -continued

Autoimmune related antigens and example LEAPS™ heteroconjugates

Seq ID No.	Disease	Protein Candidates	Abbreviation	Core epitope	Extended region	Amino acid position	J LEAPS conjugate	Ref.
947	Pernicious Anemia	H⁺K⁺ATPase	H/KATPase	EEEAEEEA	NA	35-43	NA	37.17
928		H⁺K⁺ATPase	H/KATPase	NA	NYLADEEEAEEEARVTVV	30-48	NA	56
906			J-ATPase	NA	NA	NA	DLLKNGERIEKVEGGGNYLADEEEAEE EARVTVV	
948	Autoimmune	Cytochrome p450	Cytp450-1	SLLILLLL	NA	23-31	NA	37.33
929	Hepatitis		Cytp450-1	NA	ILQVTSLILLLLLIKAAQ	18-36	NA	57
907			J-p450	NA	NA	NA	DLLKNGERIEKVEGGGILQVTSLLILL LLLIKAAQ	
930	Systemic Lupus	RNA polymerase	RNAp.1	PGGYFIVKG	NA	106-115	NA	37.7.1
931	erythematosus		RNAp.1	NA	ECPLDPGGYFIVKGVEKVI	101-120	NA	58
908			J-RNAP.1	NA	NA	NA	DLLKNGERIEKVEGGGECPLDPGGYFI VKGVEKVI	
949	Systemic Lupus	RNA polymerase	RNAP.2	GEMERDCLI	NA	990-998	NA	37.7.1
932	erythematosus		RNAP.2	NA	GGLRLGEMERDCLIGYGAS	985-1003	NA	58
909			J-RNAP.2	NA	NA	NA	DLLKNGERIEKVEGGGGLRLGEMERD CLIGYGAS	
933	Systemic Lupus	Histone	Hist1	APAAPAAPA	NA	6-13	NA	37.7.2
934	erythematosus		Hist1	NA	MSETAPAAPAAPAPAEKT	1-18	NA	59
910			J-Hist1	NA	NA	NA	DLLKNGERIEKVEGGGMSETAPAAPAA PAPAEKT	
935	Systemic Lupus	anRNPs	anRNP.1	GGRGGGGGG	NA	195-203	NA	37.7.3
936	erythematosus		anRNP.1	NA	GGGFRGGRGGGGGGFRGGGR	190-208	NA	60
911			J-RNO1	NA	NA	NA	DLLKNGERIEKVEGGGGGFRGGRGGRGGG GGGFRGGGR	
937	Rheumatic Fever	Myosin	My2	LDSKSLKI	NA	794-802	NA	37.20
938			My2	NA	IPKNLLDSKSLKIISMTL	789-807	NA	61
912			J-My2	NA	NA	NA	DLLKNGERIEKVEGGGIPKNLLDSKSL KIISMTL	
939	Rheumatic Fever	Myosin	My3	NRIIHRDVK	NA	150-158	NA	37.20
940			My3	NA	QHLHNNRIIHRDVKGNNIL	145-163	NA	62
913			J-My3	NA	NA	NA	DLLKNGERIEKVEGGGIQHLHNNRIIH RDVKGNNIL	
941	Graves disease	Thyroid hormone	T4T	SKSRSRSRS	NA	28-36	NA	37.13
942			T4T	NA	RSRSFSKSRSRSRSLSRSR	23-41	NA	63
914			J-T4R	NA	NA	NA	DLLKNGERIEKVEGGGIRSRSFSKSRS RSRSLSRSR	

TABLE 1 -continued

Autoimmune related antigens and example LEAPS™ heteroconjugates

Seq ID No.	Disease	Protein Candidates	Abbreviation	Core epitope	Extended region	Amino acid position	J LEAPS conjugate	Ref.
943	Systemic sclerosis	DNA topoisomerase	DNAtp1	GGKDAASPR	NA	812-820	NA	37.15
944				NA	GTRLHGGKDAASPRYIFTM	807-825	NA	64
915			J-DNAtp1	NA	NA	NA	DLLKNGERIEKVEGGGIGTRLHGGKDA ASPRYIFTM	
945	Goodoature's syndrome	Type IV Collaagen	C-IVp1	GAVGPAGPP	NA	192-200	NA	37.18
946				NA	APGFPGAVGPAGPPGLQGP	187-205	NA	65
916			J-CIVp1	NA	NA	NA	DLLKNGERIEKVEGGGIAPGFPGAVGP AGPPGLQGP	

Alternatively, the invention contemplates a variable immunomodulatory peptide construct having the Formula (III)

$$P_3\text{-}x\text{-}P_4 \qquad\qquad (III)$$
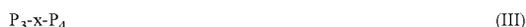
where P3 is a peptide construct comprised of X1 to X14 said peptide P3 being associated with an antigen from Table 1, and P4 is a peptide construct comprised of X1 to X14 causing a Th1 directed immune response by said set or subset of T cells to which the peptide P3 is attached or which binds to a dendritic cell or T cell receptor causing said set or subset of DC or T cells to which the peptide P3 is attached to initiate and complete, an immune response.

Alternatively, the invention contemplates a variable immunomodulatory peptide construct having the formula (IV)

$$P_5\text{-}x\text{-}P_6 \qquad\qquad (IV)$$

where P_5 is a peptide construct comprised of X_1 to X_{14} said peptide P_5 being associated with an antigen from Table 1, and P_6 is a peptide construct comprised of X_1 to X_{14} causing a T_h1 directed immune response by said set or subset of T cells to which the peptide P_5 is attached or which binds to a T cell receptor causing said set or subset of T cells to which the peptide P_5 is attached to initiate an immune response, such that X_1 to X_{10} and X_{14} describe a group of amino acids based on their features and X_{11} to X_{13} describe modifications to the peptide construct, wherein

X_1 is selected from the group consisting of Ala and Gly,
X_2 is selected from the group consisting of Asp and Glu,
X_3 is selected from the group consisting of Ile, Leu and Val,
X_4 is selected from the group consisting of Lys, Arg and His,
X_5 is selected from the group consisting of Cys and Ser,
X_6 is selected from the group consisting of Phe, Trp and Tyr,
X_7 is selected from the group consisting of Phe and Pro,
X_8 is selected from the group consisting of Met and Nle,
X_9 is selected from the group consisting of Asn and Gln,
X_{10} is selected from the group consisting of Thr and Ser,
X_{11} is $Gaba^Z$ where X_2X_3, X_3X_2, X_2X_3, X_3X_2, X_3X_3, or X_2X_2 can be substituted with X_{11};
X_{12} is selected from the group consisting of acetyl, propionyl group, D glycine, D alanine and cyclohexylalanine;
X_{13} is 5-aminopentanoic where any combination of 3 to 4 amino acids of X_2 and X_3 can be replaced with X_{13};
X_{14} is selected from the group consisting of X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9 and X_{10}; and
x is a direct bond or linker for covalently bonding P_5 and P_6. For example, a variable immunomodulatory peptide construct of formulae (III)-(IV) can contain a peptide causing a T_h2 directed immune response related to peptide J (SEQ ID No. 49), such as $X_2X_3X_3X_4X_9X_1X_2X_4X_3X_2X_4X_3X_2$ (SEQ ID No. 1091). One having skill in the art would recognize that each of X_1 to X_{14} represents a group of amino acids having similar

charge, polarity, hydrophobicity, chemical functionality, size and/or shape. As such, one having skill in the art will recognize that a variable immunomodulatory peptide can be identified by substituting a specific amino acid residue in any sequence disclosed herein with the corresponding group X_1 to X_{14} including and representing the properties of that specific residue. For example, one having skill in the art will recognized that a Gly residue can be represented by group X_1, and Trp residue can be represented by X_6 and an Arg residue can be represented by group X_4. As such, one having skill in the art will be able to unambiguously assign the tripeptide GWR as $X_1X_6X_4$. Similarly, one having skill in the art will be able to unambiguously assign any of the sequences disclosed herein to a variable immunomodulatory peptide construct having residue represented by X_1 to X_{14}. For peptide ENVGSNK (SEQ ID No. 59) can be converted to the variable immunomodulatory peptide $X_2X_9X_3X_1X_8X_9X_4$ (SEQ ID No. 60) that can be incorporated into an immunomodulatory peptide construct.

Table 2 shows exemplary antigens that can be employed as peptide P_2 in certain embodiments for the treatment of cancer. LEAPS™ heteroconjugates consistent with Formulae (I) and (II) can be formed by combining any permutation of ICBL peptide including CEL-1000. Peptide J and/or Peptide G (Peptides P_1) with an antigen peptide (Peptide P_2) as presented in Table 2. Table 2 lists antigen sequences grouped by class of cancer that such sequences are associated with. Specifically, the first column of Table 2 list the SEQ ID No. for the sequence presented in each row. The second column lists the disease or cancer for which the sequencers presented in each row relate. The third column specifies the protein from which individual amino acid sequences are derived. The fourth column gives the abbreviation for which the sequence presented in each row can be referred to. For example, AFP stands for alpha fetoprotein, whereas J-AFP indicates a LEAPS™ heteroconjugate having the ICBL Peptide J linked to AFP. Also provided on Table 2 are example LEAPS™ heteroconjugates where Peptide P_1 is Peptide J (SEQ ID No. 49) combined with an antigen Peptide P_2. The fifth column specifies the core epitope sequence, if any, for the protein described in each row, and the sixth column specifies an extended epitope sequence associated with the protein described in each row or alternatively a LEAPS™ heteroconjugate containing Peptide J (SEQ ID No. 49). The seventh column indicates the range of amino acids from the described protein corresponding to the epitope sequence. The eighth column lists any know references describing the extended or core epitope sequences, if known. References are specified by a number corresponding to the list of references found at the end of this disclosure.

The LEAPS™ heteroconjugates presented on Table 2 are conjugates where Peptide P_1 is Peptide J (SEQ ID No. 49) combined with an antigen Peptide P_2. Those skilled in the art will recognize that other constructs can be formed substituting for Peptide P_1 and Peptide P_2 where the examples on Table 2 are merely illustrative and are not limiting.

TABLE 2

Cancer related sequences and example LEAPS™ heteroconjugates

Seq ID No.	Disease	Protein	Abbreviation	Core epitope	Extended region or LEAPS™	Position	Reference
967	Colorectal cancer	NSFL1 (p97) cofactor (p47)	NSFL1 (p97) cofactor (p47)	ASSSILINESEPTTNIQIR	NA	283-301	110
1045			J-NSFL1.1ext	NA	DLLKNGERIEKVEGGASSSILINESEPTTNIQIR	NA	
968	Colorectal cancer	ATP-binding cassette, sub family F	ATP-bcsF	FAALDEEDKEEEIIK	NA	193-209	110
1046			J-ATP-bcsFpext	NA	DLLKNGERIEKVEGGGQLVNMCMNPDPEK	NA	
969	Colorectal cancer		Nek7	QLVNMCMNPDPEK	NA	269-281	110
1047			J-Nek7ext	NA	DLLKNGERIEKVEGGGQLVNMCMNPDPEK	NA	
970	Colon cancer	carcinoembryonic antigen	CEA1	YLSGANLNL	NA	653-667	90
1013			CEA1ext	NA	PPDSSYLSGANLNLSCHSA	548-672	97
1048			J-CEA1ext	NA	DLLKINGERIEKVEGGGPPDSSYLSGANLNLSCHSA	NA	
971	Colon Cancer	carcinoembryonic antigen	CEA2	YACFVSNLATGRNNS	NA	653-667	Kobatyashi
1014			CEA2ext	NA	NNNGTYACFSNLATGRNNSIVKSI	648-672	128
1049			J-CEA2ext	NA	DLLKNGERIEKVEGGGNNNGTYACFVSNLATGR NNSIVKSI	NA	
972	Liver cancer	Alpha fetoprotein	AFP	GVALQTMKQ	NA	542-550	83
1015			AFPext	NA	DLCQAGVALQTMKQEFLIN	537-555	96
1050			J-AFPext	NA	DLLKNGERIEKVEGGGDLCQAGVALQTMKQEFLIN	NA	
973	Breast cancer	Her2/Neu protein	Her2/Neu1	KIPGSLAFL	NA	369-337	85, 88
1016			Her2Neu1ext	NA	FAGCKKIFSLAFLPESFD	364-382	98
1051			J-HerNeu1ext	NA	DLLKINGERIEKVEGGGFAGCKKIFGSLAFLPESFD	NA	
974	Breast cancer	Her2/Neu protein	Her2/Neu2	RLLQETELIV	NA	689-697	89, 88
1017			Her2Neu2ext	NA	KYTMRRLLQETELVEPLTP	684-702	98
1052			J-Her2Neu2ext	NA	DLLKNGERIEKVEGGGKYTMRRLLQETELVEPLTP	NA	
975	Breast cancer	Her2/Neu protein	Her2/Neu3	ALCRWGLLL	NA	5-13	109
1018			Her2Neu3ext	NA	MELAAALCRWGLLLALLPP	1-18	115
1053			J-Her2Neu3ext	NA	DLLKNGERIEKVEGGGMELAAALCRWGLLLALLPP	NA	
976	Breast cancer	Her2/Neu protein	Her2/Neu4	HLYQGCQVV	NA	48-56	109
1019			Her2Neu4ext	NA	LDMLRHLYQGCQVVQGNLE	43-61	115
1054			J-Her2Neu4ext	NA	DLLKNGERIEKVEGGGLDMLRHLYQGCQVVQGNLE	NA	
977	Breast cancer	Her2/Neu protein	Her2/Neu6	YLVPQQGFFC	NA	1023-1032	109
1020			Her2Neu6ext	NA	VDAEEYLVPQQGFFCPDPAP	1018-1037	116
1055			J-Her2Ne6ext	NA	DLLKNGERIEKVEGGGVDAEEYLVPQQGFFCPDPAP	NA	

TABLE 2 -continued

Cancer related sequences and example LEAPS™ heteroconjugates

Seq ID No.	Disease	Protein	Abbreviation	Core epitope	Extended region or LEAPS™	Position	Reference
978 1021 1056	Breast		Her2/Neu7 Her2Neu7ext J-Her2Neu7ext	VPIKWMALWSILRRRF NA NA	NA ADGGKVPIKWMALWSILRRRFTHQSD DLLKNGERIEKVEGGGADGGKVPIKWMALWSIL RRRFTHQSD	183-198 178-203 NA	Hilthold 120
979 1022 1057	Breast Ovarian	Mucin 1	Muc1 Muc1ext J-Muc1ext	STAPPAHGV NA NA	NA STAPPAHGVSTAPPAHGV DLLKNGERIEKVEGGGSTAPPAHGVSTAPPAHGV	NA NA NA	84 105
980 1023 1058	Breast Ovarian	Mucin 2	Muc2 Muc2ext J-Muc2ext	LLNQLQVNL NA NA	NA SDGVLLNQLQVNLPHVTA DLLKNGERIEKVEGGGSDGSVLLNQLQVNLPHVTA	467-475 462-480 NA	106
981 1024 1059	Cervical cancer	E7 protein of human papiloma virus Type 16	HPV16E7.1 J-HPV16E7.1ext	DRAHYNIVTFCCK NA NA	NA GQAEPDRAHYNIVTFCCKCDSTL DLLKNGERIEKVEGGGQAEPDRAHYNIVTFCC KCDSTL	48-60 43-65 NA	87 99
982 1025 1060	Cervical Cancer	HPV 52	HPV 52 L1.1 HPV52L1.1ext J-HPV52L1.1ext	STYKNENFK NA NA	NA EVKKESTYKNENFKEYLRH DLLKNGERIEKVEGGGEVKKESTYKNENFKEYLRH	383-391 378-396 NA	114 118
983 1026 1061	Cervical Cancer	HPV 52	L1.2 L1.2ext J-HPV52L1.2ext	SAPRTSTKK NA NA	NA KRPASSAPRT STKKKVKR DLLKNGERIEKVEGGGKRPASSAPRT STKKKVKR	516-524 511-529 NA	114 118
984 1027 1062	Cervical Cancer	HPV52	L1.3 L1.3ext J-HPV52L1.3ext	TSESQLFNK NA NA	NA SGSMVTSESQLFNKPYWLQ DLLKNGERIEKVEGGGSGSMVTSESQLFNKPYWLQ	332-340 327-345 NA	114 118
985 1028 1063	Cervical Cancer	HPV 16	HPV E6.1 HPV16E6.1ext J-HPV16E6.2ext	KTLEERVKK NA NA	NA YSLYGTLEERVKKPLSEI DLLKNGERIEKVEGGGSGSYSLYGKTLEEERVKKP LSIE	86-94 81-99 NA	114 119
986 1029 1064	Cervical Cancer		HPV E6.2 HPV16E6.2ext J-HPV16E6.2ext	RLQCVQCKK NA NA	NA SVHEIRLQCVQCKKELQRR DLLKNGERIEKVEGGGSGSVHEIRLQCVQCKKEL QRR	27-35 22-40 NA	114 119
987 1030 1065	Cervical Cancer	HPV16	HPV E6.3 HPV16E6.3ext J-HPV16E6.3ext	ILIRCIICQ NA NA	NA KPLSEILIRCIICQTPLCP DLLKNGERIEKVEGGGSKPLSEILIRCIICQTPLCP	99-107 94-112 NA	114 119

TABLE 2 -continued

Cancer related sequences and example LEAPS™ heteroconjugates

Seq ID No.	Disease	Protein	Abbreviation	Core epitope	Extended region or LEAPS™	Position	Reference
988	Cervical Cancer	HPV16	HPV E7.2	YMLDLQPETT	NA	11-20	
1031			HVP16E7.2ext	NA	PTHEYMLDLQPETTDLYCY	6-25	
1066			J-HPV16E7.2est	NA	DLLKNGERIEKVEGGGPTLHEYMLDLQPETTDLYCY	NA	121
989	Several	Oncofetal antigen immature lamin receptor	OFA-ILR1	LIAAARAIVAI	NA	59-68	93
1032			OFA-ILR1ext	NA	TWEKLLLAARAIVAIENPAD	54-73	100
1067			J-OFA-ILR1ext	NA	DLLKNGERIEKVEGGGTWEKLLLAARAIVAIENPAD	NA	
990	Several	Oncofetal antigen immature lamin receptor	OFA-ILR2	ALCNTDSPL	NA	146-154	93
1033			OFA-ILR2ext	NA	NLPTIALCNTDSPLRYVDI	141-159	100
1068			J-OFA-ILR2ext	NA	DLLKNGERIEKVEGGGNLPTIALCNTDSPLRYVDI	NA	
991	Prostate Cancer	Prostatic acid phosphatase	PAP1	ILLWQPIPV	NA	135-143	91
1034			PAP1ext	NA	SIWNPILLWQPIPVHTVPL	130-148	103
1069			J-PAP1ext	NA	DLLKNGERIEKVEGGGSIWNPILLWQPIPVHTVPL	NA	
992	Prostate Cancer	Prostate Specific Antigen	PSA1	KLQCVDLHV	NA	146-154	91
1035			PSA1ext	NA	FLTPKKLQCVDLHVISNDV	141-159	104
1070			J-PSA1ext	NA	DLLKNGERIEKVEGGGFLTPKKLQCVDLHVISNDV	NA	
993	Prostate		PSA Peptide2	FLRPGDSSHDLNLLR	NA	110-125	113
1071			J-PSA1	NA	DLLKNGERIEKVEGGGFLRPGDDSSHDLNLLR	NA	
994	Prostate		PAP2	FQELESETLKSEEFQK	NA	164-179	113
1072			J-PAP2ext	NA	DLLKNGERIEKVEGGGFQELESETLKSEEFQK	NA	
995	Prostate		Periostin pep	GLESNVNVELLNALHSHMNKR	NA	152-173	113
1073			J-Perpep	NA	DLLKNGERIEKVEGGGLESNVNVELLNALHSHMNKR	NA	
996	Melanoma	Melanoma-associated antigen 2	MA2	REPVTKAEML	NA	127-136	91, 95
1036			MA2ext	NA	LLKYRAREPVTKAEMLGSVVGNWQ	122-141	101
1074			J-MA2ext	NA	DLLKNGERIEKVEGGGLLKYRAREPVTKAEMLG SVVGNWQ	NA	
997	Melanoma	Melanoma-associated antigen 3	MA3	EVDPIGHLY	NA	168-176	86, 82
1037			MA3 ext	NA	GIELMEADPIGHLYIPATC	163-181	102
1075			J-MA3ext	NA	DLLKNGERIEKVEGGGIELMEADPIGHLYIPATC	NA	
998	Melanoma	Tyrosinase-Related Protein 2	TRP2	SVVDFFVWL	NA	180-188	92
1038			TRP2ext	NA	FANCSSVYDFFVWLHYYSV	175-193	107
1076			J-TRP2ext	NA	DLLKNGERIEKVEGGGFANCSSVYDFFVWLHYYSV	NA	
999	Melanoma	Tyrosinase-Related Protein 2	TRP1	MSLQRQFLR	NA	NA	108
1039			TRP1ext	NA	MSLQRQFLRTQLWD	NA	108
1077			J-TRP1ext	NA	DLLKNGERIEKVEGGGMSLQRQFLRTQLWD	NA	

TABLE 2 -continued

Cancer related sequences and example LEAPS™ heteroconjugates

Seq ID No.	Disease	Protein	Abbreviation	Core epitope	Extended region or LEAPS™	Position	Reference
1000 1040 1078	Lung cancer	C3dg Complement	C3dg1 C3dg1ext J-C3dg1ext	AGDFLEANYMNLQR NA NA	NA GSITAGDFLEANYMNLQRSYTVA DLLKNGERIEKVEGGGSITKAGDFLEANYMNLQR SYTVA	1172-1185 1167-1190 NA	111 117
1001 1079	Lung cancer	C3dg Complement	C3dg2ext Complement J-C3dg2ext	ILLQGTPVAQMTEDAVDAER NA	NA DLLKNGERIEKVEGGGILLQGTPVAQMTEDAVDAER	980-999 NA	111, 117
1002 1041 1080	Lung cancer	C3dg Complement	C3dg3 C3dg3ext J-C3dg3ext	KGYTQQLAFR NA NA	NA LELIKKGYTQQLAFRQPSSA DLLKNGERIEKVEGGGKGYTQQLAFR	1051-1060 1046-1065 NA	111 117
1003 1042 1081	Lung cancer	C3dg Complement	C3dg4 C3dg4ext J-C3dg4ext	QPSSAAFAFVKR NA NA	NA QLAFRQPSSAAFAFVKRAPSTW DLLKNGERIEKVEGGGQLAFRQPSSAFAAFVKRA PSTW	1061-1072 1056-1077 NA	111 117
1004 1043 1082	Lung cancer	C3dg Complement	C3dg4 C3dg4ext J-C3dg4ext	WLNEQR NA NA	NA PPVVRWLNEQRYYGGG DLLKNGERIEKVEGGGPPVVRMLNEQRYYGGG	1255-1260 1250-1265 NA	111 117
1005 1083	Lung cancer		C9 PEPTIDE a J-C9a	FTPTENKAEQCCEETASSISLHG NA	NA DLLKNGERIEKVEGGGFTPTENKAEQCCEETAS SISLHG	243-267 NA	111, 129
1006 1084	Lung cancer		C9 PEPTIDE b J-C9b	QYTGTSYDPELTESSGSASHIDC NA	NA DLLKNGERIEKVEGGGQYTGTSYDPELTESSGSA SHIDC	22-44 NA	111, 129
1007 1085	Gastric (Stomach) Cancer	Pepsinogen	pep1 J-Pep1	FLKKHNLNPARKYFPQW NA	NA DLLKNGERIEKVEGGGFLKKHNLNPARKYFPQW	41-57 NA	111, 130
1008 1086	Gastric (Stomach) Cancer	Pepsinogen	pep2 J-Pep2	FLKKHLNPARKYFPQWEA NA	NA DLLKNGERIEKVEGGGFLKKHLNPARKYFPQWEA	41-59 NA	111, 130
1009 1087	Gastric (Stomach) Cancer	Leucine zipper protein	LZpf J-LZpf	ETKKTEDRFVPSSSKSEGKKSR EQPSVLSR NA	NA DLLKNGERIEKVEGGGETKKTEDRFVPSSSKSEG KKSREQPSVLSR	447-476 NA	112, 131
1010	Gastric (Stomach) Cancer	albumin fragment		DAHKSEVAHRFKDLGEENFKA LVL	NA	25-48	112, 132

TABLE 2 -continued

Cancer related sequences and example LEAPS ™ heteroconjugates

Seq ID No.	Disease	Protein	Abbreviation	Core epitope	Extended region or LEAPS ™	Position	Reference
1088			J-Albfrag	NA	DLLKNGERIEKVEGGGDAHKSEVAHRFKDLGEENFKALVL	NA	
1011	Gastric (Stomach) Cancer	alantitryposin	alTry1	SIPPEVKFNKPFVFLIEQNTKSPLFMGKVVNPTQK	NA	369-404	112, 132
1089			J-alTry1	NA	DLLKNGERIEKVEGGGSIPPEVKFNKPFVFLIEQNTKSPLFMGKVVNPTQK	NA	
1012	Testes Tumor		NY-ESQ1	LSLLMWITQCFLPVFLA	NA	156-172	Zeng, 134
1044			NY-ESQ11	NA	SCLQQLSLLMWITQCLFLPVFLAQPPSG	151-177	134
1090			J-ESQ1	NA	DLLKNGERIEKVEGGGSCLQQLSLLMWITQCLFLP VFLAQPPSG	NA	

Reversal Sequences

Embodiments also contemplate reversal sequences where the order of amino acids in Peptides P_1, P_2, P_3, P_4, P_5 and P_6 is reversed from N-term to C-terminus. For example peptide J has the sequence DLLKNGERIEKVE (SEQ ID No. 49). The reversal sequence of SEQ ID No. 49 has the sequence from N-terminus to C-terminus of EVKEIREGNKLLD. The reversal sequence for any ICBL disclosed herein is envisioned for inclusion in a LEAPS™ heteroconjugate as described herein. Further, the non-reversal sequence for an ICBL can be conjugated with an antigen sequence from Table 1 or with a reversal antigen sequence from Table. The reversal sequence for SEQ ID No. 1 is EGKPGQEGKFGAI, such that LEAPS™ heteroconjugates contemplated in certain embodiments include DLLKNGERIEKVEGGGEGK-PGQEGKFGAI and DLLKNGERIEKVEGGGIAGFK-GEQGPKGE, were Peptide J (SEQ ID No. 49) is conjugate with the reversal sequence of SEQ ID No. 1 or the non-reversal sequence of SEQ ID No. 1, respectively. Further, the reversal sequence for an ICBL can be conjugated with an antigen sequence from Table 1 or with a reversal antigen sequence from Table 1. Such LEAPS™ heteroconjugates contemplated in certain embodiments include EVKEIREG-NKLLDGGGEGKPGQEGKFGAI and EVKEIREGNKLL-DGGGIAGFKGEQGPKGE, where the reversal sequence for Peptide J (SEQ ID No. 49) is conjugate with the reversal sequence of SEQ ID No. 1 or the non-reversal sequence of SEQ ID No. 1, respectively.

Methods of Treating a Subject with LEAPS™ Heteroconjugates

Any of the LEAPS™ heteroconjugates in accordance with Formulae (I)-(IV) described above can be combined with an appropriate pharmaceutically suitable carrier with one or more optional adjuvants for administration to a subject. Such combination of the LEAPS™ heteroconjugates and a pharmaceutically suitable carrier can function to modulate an immune response as outlined for any of the autoimmune related conditions on Table 1 or can function as a vaccine to confer immune resistance to a broad spectrum of cancers as outline in Table 2. For example, an immunomodulatory peptide constructs having any of SEQ ID No. 291, 293, 315, 317, 319, 325, 331, 339, 341, 347, 355, 782, 786, 804, 828, 856-866, 867-879, 881, 883-884, 886-895, 897, 899-904, 906-916 and 964-966 or a variant thereof or another LEAPS™ heteroconjugate containing any of the described Peptides P_1 and an antigen sequence from Table 1, individually or as a mixture thereof, can be combined with a pharmaceutically suitable carrier to form a composition for modulating an immune response. Similarly, an immunomodulatory peptide constructs having any of SEQ ID No.'s 1045-1090 or a variant thereof or another LEAPS™ heteroconjugate containing any of the described Peptides P_1 and an antigen sequence from Table 2, individually, or as a mixture thereof, can be combined with a pharmaceutically suitable carrier to form a vaccine composition.

LEAPS™ technology directly mimics cell to cell interactions on the dendritic and T-cell surfaces using synthetic peptides. The LEAPS™ heteroconjugates containing the antigenic epitope linked to an ICBL can be manufactured by peptide synthesis or by covalently linking two peptides. Depending on the type of LEAPS™ heteroconjugates and ICBL used, the peptide construct is able to direct the outcome of the immune response towards the development of T-Cell function with primary effector T-cell functions: T Lymphocyte: helper/effector T Lymphocyte, type 1 or 2

(Th1 or Th2), cytotoxic (Tc) or suppressor (Ts) without excessive amounts of proinflammatory and inflammatory cytokines.

The type of the immune response elicited against an immunogen or a natural infection can be classified as Th1/Tc1, Th2/Tc2 or Th3 based on the predominant IgG subtype, the cytokines that are induced, or the presence or absence of delayed type hypersensitivity (DTH) response. A Th0 response is an earlier response that can mature into either a Th1 or a Th2 response and has features of both. The Th1 (CD4)/Tc1 (CD8) response is characterized by activation of CD4$^+$ and CD8$^+$ T cells to produce IL-2, TNF-β, and IFN-γ and to promote the production of IgM and specific IgG antibody subtypes and cell-mediated immune responses including delayed-type hypersensitivity (DTH). These response reinforce early, local and inflammatory responses. Th2 responses promote different IgG subclasses, IgE and IgA responses but not cell mediated responses to antigen (Ag). Th2 responses prevent the onset of protective Th1 cell mediated responses important for infection control, which may exacerbate disease. Initiation of Th1 and Th2 responses has important implications in terms of resistance and susceptibility to disease. Th1-dominated responses are potentially effective in eradicating infectious agents, especially viruses and intracellular infections, and are important for the induction of cytotoxic T lymphocytes (CTL). In contrast, a Th2 response is insufficient to protect against challenge with intracellular infections, but can provide protection against parasite and extracellular agents that can be neutralized by antibodies and against autoimmunity. Most importantly, for many vaccine it is thought that initiation of immunity with a Th1 response and then progression to a Th2 response promotes better immune memory.

Many suitable pharmaceutical carriers are known to persons, skilled in the art. The primary function of the pharmaceutical carrier is to assist in the delivery and/or administration the immunomodulatory peptide construct to a subject. The pharmaceutical carrier can be as simple as sterilized water. In certain embodiments, the pharmaceutical suitable carrier is a sterile pyrogen-free formulation containing from about 0.2 mg/mL to about 10 mg/mL of the immunomodulatory peptide construct in phosphate-buffered saline (PBS) and trehalose or other sugar that has been lyophilized to remove water and reconstituted prior to use with sterilized water for injection to a subject.

Optional adjuvants include products such as GMP products including Montanide ISA-51 (Seppic, Fairfield, N.J.), Depovax, a patented liposomal adjuvant currently in phase I trials by Immunovaccine Technologies, and MASI, a proprietary water-in-oil GMP adjuvant from MerciaPharma currently in phase II clinical studies. Alum is currently the only FDA licensed adjuvant of the group. In certain embodiments, the composition administered to a subject containing a LEAPS™ heteroconjugate, as described herein, has mixture of an aqueous phase and an adjuvant oil phase from about 1:4 to about 4:1.

A composition having the LEAPS™ heteroconjugate and a suitable pharmaceutical carrier with or without an optional adjuvant can be administered to a subject by subcutaneous or intramuscular injection in a therapeutically effective amount. The subject can be a mammal subject including a human subject.

Freund's Incomplete Adjuvant is also contemplated (Sigma Corp., St. Louis, Mo.). For Product Number F5506, the Storage Temperature is 2-8° C. where F5881 is a clear, amber liquid containing particulate matter (dried cells). F5506 is a clear amber liquid. Freund's incomplete Adjuvant

53

is one of the most commonly used adjuvants in research. It is used as a water-in-oil emulsion. It is prepared from non-metabolizable oils (paraffin oil and mannide monooleate). First developed by Jules Freund in the 1940's, Freund's Adjuvant is designed to provide continuous release of antigens necessary for stimulating a strong, persistent immune response. The main disadvantage of Freund's Adjuvant is that it can cause granulomas, inflammation at the inoculation site and lesions. To minimize side-effects, Incomplete Freund's Adjuvant is used for the boosts. (Freund, J. and McDermott, K., Proc. Soc. Exp. Biol. Med., 1942: 49:548-553; Freund, J. Ann. Rev. Microbiol., 1947: 1:291; Freund, J., Adv. Tuberc. Res., 1956: 7:130; Bennett, B. et al., J. Immuno. Meth., 1992: 153:31-40; Deeb, B. J. et al., J. Immuno. Meth., 1992: 152:105-113; Harlow, E. and Lane, D., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988).

Maturation of Dendritic Cells with LEAPS™ Heteroconjugates

In certain embodiments, a subject's immune response is modulated by being administered DCs matured and activated in the presence of a LEAPS™ heteroconjugate consistent with Formulae (I) or (II), or alternatively a LEAPS™ heteroconjugate consistent with Formulae (III) or (IV). Modulation of an immune response required mimicking nature's approach to recognition of antigens. DCs play a major role in initiating and directing the immune response to an antigen. The initial host response to an antigen requires internalization of the antigen into the DC followed by processing and presentation by the MHC I or II proteins for T cell recognition. DCs, macrophages and B cells are capable of presenting antigens to CD4$^+$ helper T cells and CD8$^+$ cytotoxic T cells as peptides held within grooves of the class II and I MHC proteins, respectively. These cells can be functionally divided into DC1 and DC2 cell types based on the means of their activation, their cytokine output and the nature of their influence on T cells. DC1 cells product IL12 and promote Th1-type responses whereas DC2 cells promote Th2-type responses.

As described above, the LEAPS™ heteroconjugates described herein can be administered to a subject to modulate an immune response in vivo. The immunomodulatory peptide constructs can also modulate the properties of immune cells, including DCs, ex vivo, in order to activate, mature, and direct the character and phenotype of immune cells, (e.g. DCs) contacted with the LEAPS™ heteroconjugate. Such treated immune cells, which can include DCs and/or monocytes, can then be introduced into a subject for the purpose of modulating an immune response in an autoimmune condition, DCs treated with a LEAPS™ heteroconjugate ex vivo and transferred to a subject can confer acquired immunity to the subject. As used herein, the term ex vivo means that the LEAPS™ heteroconjugate is contacted with living cells outside of the subject's body.

In certain embodiments, DCs and/or monocytes are extracted from a subject or donor away from other tissues of the body. The dendritic cells and/or monocytes are then contacted with one or more LEAPS™ heteroconjugates. DCs and/or monocytes isolated from a subject or donor can be in an immature state characteristic of immune cells prior to contact with an antigen. Such cells are herein referred to as immature DCs (iDCs). The DCs and/or monocytes can be isolated from blood derived monocytes and/or bone marrow taken from a subject or donor. DCs and/or monocytes expressly include monocyte cells capable of differentiating into macrophages and/or dendritic cells that can function to present antigens to T cells under appropriate conditions.

54

Upon contact or treatment of the isolated dendritic cells and/or monocytes with the LEAPS™ heteroconjugates, the dendritic cells and/or monocytes undergo a maturation to a state that directs and/or modulates an immune response.

As defined herein, the term "immature DCs" (iDCs) refers to cells derived from a donor or subject that are not competent to induce T cell activation upon interaction with T cells. Such iDCs are also known in the art as naïve DCs. Such iDCs can have certain physical characteristics such as a reduced level of expression of CD80 and/or CD86, MHC molecules (class I and/or class II), other surface markers and a reduced appearance of dendrites. Immature DCs, as defined herein, expressly includes monocytes that can be stimulated to form dendritic cells. As defined herein, terms "matured DCs" and "more matured DCs" refer to DCs after contact with any of the LEAPS™ heteroconjugates described herein. Such matured DCs can have certain physical characteristics including upregulation of CD80 and/or CD86, MHC I or II molecules, an increased appearance of dendrites and secretion of IL-12p70.

In certain embodiments, contacting monocyte cells with one or more LEAPS™ heteroconjugates can induce the development of the monocyte cells or iDCs to DC1 (Th1-inducing dendritic cells) and/or DC2 cells (Th2-inducing dendritic cells) or other cell type allowing for acquired immunity when transferred to a subject. In certain other embodiments, iDCs and/or monocytes isolated from a subject or donor are contacted with a media containing granulocyte-macrophage colony stimulating factor (GM-CSF) to stimulate the expression of CD11c on the surface of the iDCs or monocytes. The iDCs and/or monocytes after exposure to GM-CSF are contacted with one or more LEAPS™ heteroconjugates to induce the maturation of the monocyte cells or iDCs to DC1 (Th1-inducing dendritic cells) and/or DC2 cells (Th2-inducing dendritic cells) or other cell type allowing for an immune response to be modulated when administered to a subject.

In certain embodiments, DCs and/or monocytes are extracted from a subject or donor away from other tissues of the body in a composition of isolated iDCs and/or monocytes. As described herein, a composition of isolated DCs and/or monocytes is composition in which the DCs and/or monocytes are present away from other body tissue including blood or bone marrow. In certain embodiments, a composition of isolated DCs and/or monocytes contains at least 50% of the viable cells present in the composition being DCs and/or monocytes. In certain embodiments, the composition of DCs and/or monocytes is substantially free from whole red blood cells.

In certain embodiments, the iDCs and/or monocytes are contacted or treated with one or more LEAPS™ heteroconjugates for about 6 hours to about 96 hours or from about 12 hours to about 72 hours. In certain other embodiments, the iDCs and/or monocytes are contacted or treated with one or more LEAPS™ heteroconjugates for a period of time longer than about 6 hours. In additional embodiments, the iDCs and/or monocytes are contacted or treated with one or more LEAPS™ heteroconjugates for a period of time longer than about 12 hours. In certain additional embodiments, the iDCs and/or monocytes are contacted or treated with one or more LEAPS™ heteroconjugates for a period of time longer than about 24 hours. In certain embodiments, the iDCs and/or monocytes are contacted or treated with one or more LEAPS™ heteroconjugates at a ratio from about 5 to about 50 micromoles of one or more LEAPS™ heteroconjugates per 10^6 iDCs and/or monocytes. In certain other embodiments, the iDCs and/or monocytes are contacted or treated

with one or more LEAPS™ heteroconjugates at a ratio greater than about 5 micromoles of one or more LEAPS™ heteroconjugates per 10^6 iDCs and/or monocytes.

In certain embodiments, the iDCs and/or monocytes are contacted with GM-CSF for a period of about 1 day to about 10 days or from about 3 days to about 10 days. In certain additional embodiments, the iDCs and/or monocytes are contacted with GM-CSF for a period greater than about 5 days. In certain embodiments, the iDCs and/or monocytes are contacted with a media having a concentration of from about 5 to about 200 ng/mL of GM-CSF or from about 10 to about 150 ng/mL or GM-CSF. In other embodiments, the DCs and/or monocytes are contracted with a media having a concentration of GM-CSF greater than about 5 ng/mL. In certain embodiments, the DCs and/or monocytes are contacted with a media having a concentration of GM-CSF greater than about 15 ng/mL of GM-CSF.

Upon contact of iDCs and/or monocytes with the LEAPS™ heteroconjugate, an increased expression level of interleukin-12p70 (IL-12p70) can be observed relative to iDCs and/or monocytes not contacted with the LEAPS™ heteroconjugate. In certain embodiments, iDCs and/or monocytes contacted with the LEAPS™ heteroconjugate exhibit an up-regulation of at least one of the following: CD80, CD86, MHC class I, or MHC class II cell surface markers relative to iDCs and/or monocytes not contacted with the LEAPS™ heteroconjugate.

Immature dendritic cells and/or monocytes after contact with an immunomodulatory LEAPS™ heteroconjugate can be referred to as matured dendritic cells. The matured dendritic cells can modulate an immune response when administered or introduced into a subject. An immune response can be induced in a subject under situations where matured dendritic cells are washed free of LEAPS™ heteroconjugate that is unbound from the surface of a dendritic cell. As such, the amount of any antigen, including the antigen peptide P_2 forming part of the LEAPS™ heteroconjugate, introduced into a subject is limited.

Without wishing to be bound by any one particular theory, it is believed that the LEAPS™ heteroconjugate is retained on the surface of DCs in a manner allowing for the interaction of the LEAPS™ heteroconjugate with T cell receptor present on the surface of T cells. As such, DCs matured in the manner described above can be introduced or administered to a subject such that the LEAPS™ heteroconjugate present of the surface of the introduced or administered DCs can interact with the subject's in situ T cells to direct and/or modulate an antigen specific immune response. More specifically, activation of T cell-mediated immune response requires multiple stimulator interactions, including interaction with T cell receptor (TCR) present on the surface of T cells. It is believed that these more matured DCs having the LEAPS™ heteroconjugate present of the surface can provide the necessary interaction to activate T cells and direct an immune response to the Peptide P_2 antigen of the LEAPS™ heteroconjugate. Further, it is believed that these more matured DCs formed using the methods described herein have an advantageous profile of secreted cytokines that do not stimulate a cytokine storm or other delirious inflammation response in a subject.

The more matured dendritic cells and/or T cells can be used in an autologous fashion. In certain embodiments, iDCs and/or monocytes are isolated from a subject to be treated, such isolated cells can be blood derived monocytes and/or bone marrow cells taken from the subject. The isolated iDCs and/or monocytes are contacted with one or more LEAPS™ heteroconjugates having the structure P_1-x-P_2 or P_2-x-P_1 to induce maturation to more matured dendritic cells. An effective amount of the DCs are administered to the same subject from where the matured cells were originally isolated. The subject can be a mammal, including a human.

In certain other embodiments, iDCs and/or monocytes can be isolated from a compatible donor, treated with a heteroconjugate peptide having the structure P_1-x-P_2 or P_2-x-P_1 to induce maturation to form matured dendritic cells, and an effective amount of the matured dendritic cells and/or T cells administered to a subject having compatibility with the donor.

The matured DCs having been treated with the LEAPS™ heteroconjugate having the structure P_1-x-P_2 or P_2-x-P_1 can be administered to a subject either as a prophylactic or therapeutic treatment against the development of an autoimmune condition for an antigen contained in the LEAPS™ heteroconjugate, as a prophylactic or therapeutic treatment against the development of a cancer associated with an antigen contained in the LEAPS™ heteroconjugate or to modulate an existing immune response against such an autoimmune condition or cancer present in the subject.

Diagnostic Application of Dendritic Cells with LEAPS™ Heteroconjugate

Further, it is believed that the modulation of an antigen specific immune response by maturation of DCs with the LEAPS™ heteroconjugate will sensitize the matured DCs to locate and/or to target the tissues, organ systems or other structures in the body where an autoimmune event is occurring. As such, the matured DCs can be used to detect, to diagnose and/or to locate an autoimmune related condition or cancer cells. Often, detection of autoimmune related conditions or cancer required the condition to reach a relatively advanced stage such that symptoms can be observed. Here, the matured DCs treated with a LEAPS™ heteroconjugate can be used to sensitively detect early stage conditions even before the appearance of symptoms.

Matured DCs treated with a LEAPS™ heteroconjugate have a property allowing for the location of such matured DCs to the site of an autoimmune condition or cancer cells in a subject. The LEAPS™ heteroconjugate has a P_2 peptide sequence originating or derived from an antigen associated with of an autoimmune condition or cancer, as described above. Maturation of DCs through treatment with a LEAPS™ heteroconjugate allows for the matured DCs to collect or locate in an area of a subject's body where autoimmune related conditions (e.g. arthritic joints) or cancer cells are present. As such, an autoimmune related condition or cancer can be detected by observing matured dendritic cells administered to a subject collecting, locating or concentrating at a site within the subject's body where an undesirable immune response, such as an autoimmune response, is occurring or where cancer cells are present.

In certain embodiments, matured DCs can be labelled with a tracking marker to allow for their location within a subject's body to be tracked after administration to the subject. For example, matured DCs can be labelled with radionuclides (radioisotopes) to allow for the location of the labeled, matured DCs to be detected using appropriate equipment. Appropriate radionucleotides include radioactive isotopes of iodine such as ^{131}I or ^{128}I as well as other radionuclides including ^{18}F, ^{32}P, ^{64}Cu, ^{90}Y, ^{99m}Tc, ^{124}I, ^{89}Zr, ^{111}In, ^{188}Re, or ^{177}Lu. The location of radionucleotides can be determined using a radiation detector, single-photon tomography/computed tomography (SPECT/CT), scintillation camera, position emission tomography or photographic film sensitive to radiation. In certain further embodiments, matured DCs can be labeled with a dye, such as Cy5.5,

Alexa Fluor®, carboxyfluorescein succinimidyl ester (CSFE), and other near-infrared (NIR) probes, where the presence of such dye-labelled DCs can be detected in tissues taken by biopsy from a patient administered the matured DCs. Additional NIR probes include Cy 5.5, CSFE, Alexa Fluor® dyes (Alexa), or other NIR dyes covalently linked to 4-N(S-glutathionylacetylaminophenyl)arsenoxide or 2,3-di-cyanonaphthalene, such as 4-N(S-glutathionylacetylamino-phenyl)aresenoxide-Cy5.5 4-N(S-glutathionylacetylamino-phenyl)arsenoxide-CSFE, 4-N(S-glutathionylacetylaminophenyl)arsenoxide-Alexa, 2,3-dicyanonaphthalene-Cy5.5, and 2,3-dicyanonaphthalene-CSFE, and 2,3-dicyanonaphthalene-Alexa. In further embodiments, luminescence probes can also be conjugated to the LEAPS™ peptide construct or to LEAPS™-treated DCs. In certain embodiments, the presence of fluorescence in a tissue sample taken from a subject's body is determined by flow cytometry.

The immunomodulatory LEAPS™ heteroconjugates can be used to modulate a subject's immune system to detect the presence of an autoimmune condition at an early state. The LEAPS™ heteroconjugates can be used to mature immature DCs or monocytes isolated from the subject or a compatible donor to be sensitive to a desired antigen involved in an autoimmune related condition. Since the DCs can be manipulated outside of the body, the matured DCs can be labelled with a tracking marker in a manner allowing for sensitive detection. In particular, labelling with radionu-clides can allow for detection down to very low levels.

In other embodiments, the immunomodulatory LEAPS™ heteroconjugates can be used to modulate a subject's immune system to detect the presence a cancer at an early stage. The LEAPS™ heteroconjugates can be used to mature immature DCs or monocytes isolated from the subject or a compatible donor to be sensitive to a desired antigen origi-nating or derived from cancer cells where detection is desired. As described above, the DCs can be manipulated outside of the body and labelled with a tracking marker in a manner allowing for sensitive detection. In particular, label-ling with radionuclides can allow for detection down to very low levels. Similarly, a concentrated location of a contrast-ing agent can be made readily apparent in an MRI image or the presence of a fluorescent or luminescent dye can be discerned at very low levels of such agents.

In certain embodiments, immature DCs and/or monocytes are collected from a subject or a compatible donor and matured by treatment or contact with a LEAPS™ hetero-conjugates having a structure of Formulae (I) or (II) and incorporating an antigen peptide (P2) sequence as described on Table 1. Alternatively, a LEAPS™ heteroconjugate hav-ing a structure of Formulae (III) or (IV) can also be used. The matured DCs are administered to the subject through an intravenous route or another appropriate route and a period of time is allowed to elapse. A diagnostic determination of the presence or location of an autoimmune condition can be made by observing the location of the administered matured dendritic cells and/or tracking marker. When the relevant condition is present in the body of the subject, the matured DCs and/or tracking marker will concentrate at the location, tissue type or organ structure where the autoimmune related condition is occurring or where cancer cells are present. When the targeted condition is not present, the matured DCs and/or tracking marker is expected to be diffused in different locations of the subject's body and not concentrated in any particular location, tissue type or organ structure.

The diagnostic determination can be made by only observing the location, tissue type or organ structure for

which the autoimmune condition is expected to be found. For example, if matured DCs are made with a LEAPS™ heteroconjugates containing an antigen sequence derived from collagen, then only observation of presence of the matured DCs and/or tracking marker in specific joints of the body that may be affected by arthritis needs to be made. Since the amount of tracking marker administered to the subject is known, a determination of a concentration of the matured DCs and/or tracking marker in a specific location, tissue type or organ structure can be made without the need for a direct comparison with other body tissues.

In certain embodiments, a majority of the matured DCs and/or tracking marker is present in a specific location, tissue type or organ structure of the subject indicating the presence of the targeted autoimmune condition or cancer. In certain other embodiments, at least about 75% of the matured DCs and/or tracking marker are present in a specific location, tissue type or organ structure of the subject indi-cating the presence of the targeted autoimmune condition or cancer. In certain other embodiments, less than a majority of the matured DCs and/or tracking marker present in a specific location, tissue type or organ structure can indicate the presence of the targeted autoimmune condition or cancer when the matured DCs and/or tracking marker in the specific location, tissue type or organ structure is higher than in surrounding areas.

Labeling of Dendritic Cells and Delivery of Therapeutic Compounds

The ability of LEAPS™ heteroconjugates treated DCs to locate to the site of an autoimmune event or cancer can be utilized to deliver therapeutic agents directly to the site of the autoimmune related condition or cancer in addition to the diagnostic applications discussed above. At least a portion of the LEAPS™ heteroconjugates used to treat the DCs is expected to remain associated with the DCs. As such, the LEAPS™ heteroconjugate can be associated or conju-gated to a tracking marker, such as a radioisotype, or to a therapeutic agent. For example, the LEAPS™ heteroconju-gates peptide can be conjugated to monomethyl auristatin (a microtubule inhibitor), saporin, or maytansinoid 1 molecule using a cathespsin cleavable valine-citrulline (vc) dipeptide linker to a cysteine or lysine residue on the LEAPS™ heteroconjugates. The therapeutic agent or radioisotope con-jugated to a LEAPS™ heteroconjugate or to an mAb can be conjugated or linked to a lysosomatropic agent. A lysoso-matropic agent is a weak organic base that can diffuse through membranes but will become protonated in the lysosome of a cell, where the protonated lysomatropic agent is unable to diffuse through membranes and will, therefore, be trapped within the cell. Hydrophobic amines, including butylamine, spermidine, spermine, methylamine, and cya-nine dyes (including those used for studying membrane potential or that are used as tracers in neurobiology) are examples of lysosomatropic agents. These lysosomatropic agents can be modified to be conjugated to a radioisotope or to a therapeutic compound (e.g. cytokines, SEB, SEA or other molecules) by a cleavable linkage to the radioisotope or compound. Other active sites on select amino acids can also serve as sites of attachment of drugs, dyes toxins or cytokines, such as OH groups on serine or threonine resi-dues, the CH_3S group on methionine residues, carboxyl groups (COOH) on aspartic or glutamic acid residues, amine groups on lysine residues or the N-terminus of the peptide construct or amide groups on asparagine. Such groups (e.g. OH, COOH, CH_3S amine, amide, etc) can be engineered into the LEAPS™ heteroconjugates to serve as a conjuga-tion site for the therapeutic compound or dye. In a similar

manner, a radioisotope chelated by a chelation compound can be conjugated to the LEAPS™ heteroconjugates.

For the targeting of cancer, the ability of LEAPS™ heteroconjugate treated DCs to locate to the site of cancer cells can be utilized to deliver therapeutic agents directly to the site of the cancer cells. At least a portion of the LEAPS™ heteroconjugates used to treat the DCs is expected to remain associated with the DCs. As such, the LEAPS™ heteroconjugate can be associated or conjugated to a tracking marker, such as a radioisotope, or to a therapeutic or anti-cancer agent. For example, the LEAPS™ heteroconjugates peptide can be conjugated to a radioisotope chelated by a chelation compound can be conjugated to the LEAPS™ heteroconjugates.

An alternate approach for the association of a tracking marker or a therapeutic compound with the of LEAPS™ heteroconjugate treated DCs is conjugation of such species to the cell surface of the DCs. A tracking marker or therapeutic agent can be conjugated with a monoclonal antibody (mAb), a therapeutic agent or an anticancer agent. An organic molecule having the property of a therapeutic agent, an anticancer agent or a dye can be linked to a monoclonal antibody through a cysteine, lysine or other amino acid residue present on the mAb. A cleavable linker such as a valine-citrulline dipeptide linker can also be used. The mAb can have affinity for MHC II (anti-MHC II) or CD11c (anti-CD11c) or another cell surface marker present on DCs. Examples of cell surface markers for which the antibody can have affinity to include DEC-205, Dectin-1, DC-SIGN, and DC-LAMP. In this manner, the mAb servers to associate the therapeutic agent with the DCs with high affinity. Radioisotopes can be associated with DCs in a similar manner, where a radioisotope is either chelated by an organic chelation molecule or covalently bonded to an organic molecule conjugated with a mAb.

Examples of therapeutic compounds or anticancer agents include immune system suppressors or even cytotoxic drugs such as monomethyl auristatin E (MMAE), saporin, maytansinoid 1, ozogamicin, doxorubicin, emtansine, carboplatin, 5-fluorouracil, docetaxel, gelonin, receptor tyrosine kinase inhibitors, phosphatidylinositol 3-kinase inhibitors, norifensine, and irinotecan (CPT-11) as well as larger molecules such as interferon-alpha, staphylococcal enterotoxin A superantigen (SEA) or staphylococcal enterotoxin B (SEB) (using the chemical conjugating reagent N-succinimidyl 3-(2-pyridyldithio)propionate) or other agents such as 1-Ethyl-3(3-dimethylaminopropyl)carbodiimide (EDC) or 3-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) for some dyes or therapeutic agents) or other materials. Cytokines such as interferon-α can also be used as therapeutic agents. Additional therapeutic agents include methotrexate, 5-fluorouracil, azathioprine, mecaptopurine, cyclophosphamide, cyclosporine A and prednisone. The use of LEAPS™ heteroconjugate-treated DCs to deliver therapeutic agents can be particularly effective for addressing toxicity. For example, the antimitrotic agent auristatin is 100-1000 more potent than doxorubicin making auristatin highly toxic and not well-tolerated at therapeutic doses. By targeting such cytotoxic agents by conjugation with LEAPS™ heteroconjugate-treated DCs, the delivery of the cytotoxic agents to target cells is more specific and reduces safety concerns.

Further, radioisotopes can serve as therapeutic agents as well as tracking markers. Radioisotopes such as ^{90}Y and ^{188}Re are high-energy beta-emitters that can deliver ionizing radiation to the site of autoimmune related condition. Simi-

larly, radioisotopes such as ^{64}Cu and ^{124}I are alpha-emitters that can be used to deliver ionizing radiation to the site.

In a further embodiment, the LEAPS™ heteroconjugate or a mAb can be conjugated to a fluorescent dye. Suitable dyes include N,N'-di-carboxypentyl-indodicarbocyamine-5, 5'-disulfonic acid (Cy 5.5) and other near-infrared (NIR) dyes such as those made by Alex fluor. Additional NIR probes include Cy 5.5 covalently linked to 4-N(S-glutathionylacetylaminophenyl)-arsenoxide-Cy5.5 and 2,3-dicyanonaphthalene-Cy5.5. Additionally, DCs can be directly stained by carboxyfluorescein succinimidyl ester (CFSE). Additional NIR probes include CSFE, Alexa Fluor® dyes (Alexa), or other NIR dyes covalently linked to 4-N(S-glutathionylacetylaminophenyl)arsenoxide or 2,3-dicyanonaphthalene, 4-N(S-glutathionylacetylaminophenyl)arsenoxide-CSFE, 4-N(S-glutathionylacetylaminophenyl)arsenoxide-Alexa, and 2,3-dicyanonaphthalene-CSFE, and 2,3-dicyanonaphthalene-Alexa. In particular, CY 5.5 Alexa Fluor® and other NIR dyes exhibit low absorption of the NIR signal in tissue at operating wavelengths and may be quenched by conjugation of two or more NIR probe molecules together. Cleavage of the conjugation bonds results in fluorescence dequenching and generation of a signal that is suitable for imaging. A fluorescent image can be made by endoscopy or by taking a tissue biopsy. A tissue biopsy can be examined by flow cytometry to identify the presence of fluorescent cells.

EXAMPLES

The ability of a LEAPS™ heteroconjugate containing an antigen originating from herpes simplex I virus (HSV-I) was investigated to demonstrate the ability of LEAPS™ heteroconjugate to cause the maturation of treated DCs. The JgD LEAPS™ heteroconjugate peptide contained peptide J (DLLKNGERIEKVE, SEQ ID No. 49) conjugated to a peptide from the N-terminus of HSV-I glycoprotein D (gD) (SLKMADPNRFRGKDLP, SEQ ID No. 952), amino acid 8-23, through a triglycine linker. As such, the JgD LEAPS™ heteroconjugate peptide has the sequence DLLKNGERIEK-VEGGGSLKMADPNRFRGKDLP (SEQ ID No. 953).

An additional LEAPS™ heteroconjugate containing an antigen derived from HIV virus was also used to demonstrate the ability of LEAPS™ heteroconjugate to cause a maturation of DCs. The JH LEAPS™ heteroconjugate peptide vaccine contained the peptide J (SEQ ID No. 49) ICBL conjugated to a peptide "HGP-30 (H) peptide from the p17 HIV gag protein YSVHQRIDVKDTKEALEKIEEEQNK-SKKKA (aa 85-115) (SEQ ID No. 954) through a triglycine linker. As such, the JgH LEAPS™ heteroconjugate peptide has the sequence DLLKNGERIEKVEGGGYSVHQRID-VKDTKEALEKIEEEQNKSKKKA (SEQ ID No. 955).

Preparation of Bone Marrow Cells

Bone marrow (BM) cells were prepared. Briefly, the femurs and tibias were obtained from five C57BL/6 female mice, and the ends were removed to expose the hollow bone packed with marrow. BM cells were flushed from the bones with cold Hanks Balanced Salt Solution (HBSS) using a sterile disposable 22 g needle and pooled. Red blood cells (RBCs) were lysed using Tris-buffered ammonium chloride and resultant cells were washed 3 times in HBSS, BM cells were suspended in tissue culture medium (TCM) (RPMI 1640 with glutaminic plus 100 mg/nL PenStrep, 50 μM 2-mercaptoethanol, and 5% fetal calf serum) at approximately 5×10^6 cells/ml and incubated for 1 hour at 37° C. in a 5% CO2 atmosphere in plastic tissue culture flasks to remove adherent, mature macrophages. Decanted non-ad-

herent cells were resuspended in TCM and 1.5×10^6 BM cells in 1 ml were placed into each well of a 24 well tissue culture plate (Falcon) and either left untreated or treated with 14.5 micromoles of peptide J or JgD LEAPS™ heteroconjugate. After incubation for 48 hrs at 37° C., cells were viewed and photographed for changes in morphology, tissue culture supernatants were removed and the cells were prepared for flow cytometric analysis.

Immature DCs were generated from the bone marrow of five normal C57BL/6 female mice. Briefly, BM cells were harvested as before and cultured at 5×10^5/ml in 75 cm2 flasks at 37° C., 10% CO2 for 6 days in a complete media (CM) containing RPMI 1640, 10% fetal bovine serum, 2 mM glutamine, 0.1 mM nonessential amino acids, 100 units/ml sodium pyruvate, 100 mg/ml PenStrep, 0.5 mg/ml fungizone, 50 ug/ml gentamicin, 50 um 2-mercaptoethanol, supplemented with 10 ng/ml of human IL-6 (Peptrotech, Rocky Hill, N.J.) and 10 ng/ml human Flt-3 (gift of Amgen, Thousand Oaks, Calif.). On day 6, the cells were washed twice in Dulbecco's PBS. 4×10^6 cells/well were transferred to a 24-well cluster plate and cultured in CM supplemented with 10 ng/ml of human GM-CSF (gift of Immunex, Seattle, Wash.), and incubated for 24 hrs. Cells were then analyzed by flow cytometry for expression of CD11c, CD80, CD86, MHC II, CD34, and OX40L, confirming the purity or the iDC population.

Immature DCs were either untreated or treated with 3.625, 7.25, or 14.5 micromoles of JgD peptide and maintained in CM without GM-CSF. After 48 h incubation, spent medium was removed and immediately tested for the presence of IL-12p70 by direct ELISA.

Flow Cytometry Analysis

For analysis of CD11c and CD86 expression, untreated and peptide treated BM cells, prepared and treated as described above, were labeled with PE-anti-Cd11c or PE-anti CD86 (Beckman Coulter Fullerton, Calif.) At least 106 cells were analyzed (Altra FACS, Beckman Coulter) using forward and side scatter parameters to limit (gating) the immunofluorescence analysis to cells of the size and granularity of monocytes and dendritic cells.

CD3+ cells were removed from BM cells using the fluorescence activated cell sorter and then untreated or treated with JgD, gD or JH. Flow cytometric analysis of the sorted population confirmed the removal of CD3 positive cells. The CD3− BM cells were labeled with FIT C-anti-CD8 (Beckman Coulter (clone 53-6.7)), fixed with para-formaldehyde, permeabilized with saponin (Intraprep, Immunotech), labeled with PE-anti-IL-12p70 (Beckman Coulter) and then post fixed with paraformaldehyde prior to immunofluorescence analysis.

The monocyte population of BM cells, as defined by light scatter parameters, was analyzed on the second day after treatment with J, gD, JgD or JH. Representative flow cytometric results are presented in FIGS. 1A-1B.

The untreated monocyte population contained very few CD11c or CD86 positive cells whereas the JgD LEAPS™ heteroconjugate-treated cells expressed CD11c (FIG. 1A) and CD86 (FIG. 1B). CD11c is a type I transmembrane protein found on most human and mouse dendritic cells and CD86 is a cell marker for mature DCs capable of signaling and activating T cells.

Treatment with gD or the J-ICBL alone caused no discernible change in CD111c or CD86 expression. Similarly, there was no significant increase in IL-12p70 expressing cells following J-ICBL treatment.

Herpes Simplex Virus Challenge in the Zosteriform Spread Mouse Model

Mouse bone marrow cells were treated with JgD LEAPS™ heteroconjugate and incubated for 24 h, washed free of unbound vaccine or media components, and then injected subcutaneously or intraperitoneally. The subcutaneously or intraperitoneally injected cells conferred protection from disease and death from lethal herpes simplex virus challenge in the zosteriform spread mouse model. Mice (C57BL/6) received two injections of either JgD LEAPS™ heteroconjugate-treated DCs or untreated bone marrow cells. JgD LEAPS™ heteroconjugate-treated DCs were prepared by treating bone marrow cells with JgD for 24 h and the cells were washed free of peptide and media components. JgD LEAPS™ heteroconjugate-treated DCs or bone marrow cells were injected intradermally and intraperitoneally with a two week window and then the mice received a lethal challenge with HSV-I H129 in the zosteriform-challenge model. Mice were either untreated, treated with 24 h cell cultured bone marrow cells (BM). J Peptide ICBL treated bone marrow cells (J-BM), JH LEAPS™ heteroconjugate-treated dendritic cells (JH-DC) or JgD LEAPS™ heteroconjugate-treated bone marrow cells (JgD-DC). Symptoms were scored on the following scale: 0: no disease; 1: non-specific changes: 2: local disease; 3: early zosteriform spread; 4: later zosteriform spread with scores; 5: moribund disease; 6: death. Mice were scored daily for symptoms and the average for the group is presented in FIG. 3.

Mice receiving no treatment, untreated mouse bone marrow cells (BM), mouse bone marrow cells treated with the J peptide immune cell binding ligand only (J-BM) and/or JH LEAPS™ heteroconjugate-treated dendritic cells (JH-DC) incurred significant disease with zosteriform spread and death of a majority of the group within 2 weeks. Whereas, all of the mice receiving bone marrow cells treated with JgD LEAPS™ heteroconjugate-treated dendritic cells (JgD-DC) and challenged with HSV-survived and most showed signs of disease (6 of 7).

FIG. 2 shows a Kaplan Meier survival curve for the JgD-DC and untreated BM vaccinated mice. FIG. 3 is a disease score plot showing a reduction or prevention of symptoms of disease signs for mice treated with JgD-DC as compared with: No treatment; Untreated BM; j BM; and JH-DC.

These results prove that the DCs generated by JgD treatment of bone marrow cells are sufficient to initiate and develop an immune response sufficient to provide protection from a large lethal HSV infection. These results further prove that the LEAPS peptide stays on the surface of the DC for long periods and can interact with T cells to elicit the response.

Immune Modulation in an Arthritis Model

The ability of LEAPS™ heteroconjugates to modulate an immune response in an autoimmune condition was investigated for heteroconjugates labeled CEL-2000 (DLLKNGE-RIEKVEGGGTGGKPGIAGFKGEQGPKGEP, SEQ ID No. 912) and CEL-2003 (DLLKNGERIEKVEGGGD-AGEPGIAGFKGDQGPKGET, SEQ ID No. 956). CEL-2000 is formed by linking Peptide J (SEQ ID No. 49) to the human type II collagen peptide 254-273 (TGGKPGIAGFK-GEQGPKGEP, SEQ ID No. 812) through a triglycine linker. CEL-2003 is formed by linking Peptide J (SEQ ID No. 49) to the corresponding murine type II collagen peptide 254-273, DAGEPGIAGFKGDQGPKGET (SEQ ID No. 855), through a triglycine linker.

The following study is of CEL-2000 therapeutic vaccine for collagen induced arthritis (CIA) where the first step was to identify a good animal model for testing the vaccine,

which is the collagen induced arthritis (CIA) model in young (6-7) week old male DBA/IJ mice. These mice received 2 injections of bovine collagen the first in complete Freund's adjuvant (CFA) on day 0 and then 3 weeks later on day 21, in Incomplete CFA. After the second collagen injection, the mice were evaluated daily for any joint swelling or redness. Each of the paws was scored on a 4 point scale (Arthritis Index (AI) with respect to the number of digits with symptoms and the thickness of the paw measured, at least 3-4 times a week. Each mouse was weighed weekly.

When significant disease is noted, usually about day 28, the mice are grouped (n=8) with a range of scores between 1 and 6 and group mean of 2.5 to 3. At this point the therapy begins according to protocol. Controls include groups with induced disease but no therapy and groups of healthy mice without induced disease. A therapy control of Enbrel® (3 mg/kg, every other day) was included. Over the 28-day course of study, the outcomes of different CEL-2000 and CEL-2003 treatment schedules were compared with Enbrel. In this study, CEL-200 treatment with 2 doses of 33 or 100 nmol were given subcutaneously on days 0 and 7 or on days 0 and 14. CEL-2003 was used at one dose of 33 mmol on days 0 and 14. Most regimes reduced the progression of arthritis disease to levels that were at least as good as those of mice treated with Enbrel® (every other day for the 28 days of the study). Immunization of mice with the 100 nmol dose (3× treatment) on days 0 and 7 appeared to limit the progression of disease throughout the experimental period as shown in FIG. **4**. The AI score for each paw (4 paws total) were added for each group and reported on FIG. **4**.

The CEL-2003 links the corresponding murine (CII254-273 sequence DAGEPGIAGFKGDQGPKGET (SEQ ID No. 855) to the J ICBL. This trial suggests that the dosage and schedule of administration (time between initial and second immunization) are important parameters for CEL-2000 treatment. Use of a student "t" Test analysis of Treatment groups at day 7 days 14 and 21 to calculate the p value showed the 3× dose of CEL-2000 on day 0 and 14 followed by 3× on day 0 and 7 or 1× on day 0 and 7 is equivalent to days 0 and 14 and slightly better than Embrel every other day for all 28 days.

As such LEAPS™ heteroconjugates are demonstrated to be at least as affective as Enbrel® in addressing the autoimmune response in the CIA model. As such, LEAPS™ heteroconjugates are demonstrated immunomodulators.

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SEQUENCE LISTING

The patent contains a lengthy "Sequence Listing" section. A copy of the "Sequence Listing" is available in electronic form from the USPTO web site (http://seqdata.uspto.gov/?pageRequest=docDetail&DocID=US10179164B2). An electronic copy of the "Sequence Listing" will also be available from the USPTO upon request and payment of the fee set forth in 37 CFR 1.19(b)(3).

We claim:

1. A peptide for directing an immune response in a subject as a vaccine or to modulate immune response in an autoimmune disease or cancer, or for maturing dendritic cells, comprising a peptide construct having the formula P_1-x-P_2, wherein

P_2 represents a specific antigenic peptide competent for recognition by a class or subclass of immune cells or binding to an antibody;

P_1 represents an immunomodulatory peptide which is a portion of an immunoprotein capable of promoting binding to a class or subclass of immune cells and directing a subsequent immune response to the peptide P_2; and

x represents a covalent bond or a divalent linking group, wherein the peptide P_2 is derived from a cancer cell or derived from a protein involved in an autoimmune disease; and

wherein the peptide construct is selected from the group consisting of SEQ ID No's: 858, 862, 877, 881, 883-884, 907-916, 966, and 1045-1090.

2. The peptide of claim 1, wherein the peptide P_2 is selected from the group consisting of SEQ ID No's: 963 and 1024.

3. The peptide of claim 1, wherein the peptide construct is selected from the group consisting of SEQ ID No's: 966 and 1059.

4. The peptide of claim 1, wherein the peptide P_2 is derived from a protein expressed by a cancer cell.

5. The peptide of claim 1, wherein the divalent linker comprises one or more glycine residues.

6. A method for targeting matured dendritic cells to a site of one or more cancer cells in a subject or a site of an autoimmune process in a subject, comprising:

contacting immature dendritic cells or monocytes with a peptide construct ex vivo under conditions suitable for maturation of the cells to form the matured dendritic cells; and

administering an effective amount of the matured dendritic cells to the subject, wherein a majority of the matured dendritic cells administered to the subject locate to the site of one or more cancer cells or the site of an autoimmune process,

wherein the matured dendritic cells are labeled with a tracking marker allowing for detection of the matured dendritic cells; and

wherein the peptide construct is the peptide of claim 1.

7. The method of claim 6, wherein the site of one or more cancer cells is detected in the subject by observing the matured dendritic cells concentrated in a location, tissue type or organ structure of the subject's body.

8. The method of claim 6, wherein the tracking marker is selected from the group consisting of a radionuclide, a luminescence dye and a fluorescent dye.

9. The method of claim 6, wherein the immature dendritic cells or monocytes are collected from the subject, and where the cells after maturation are introduced back into the subject in an autologous fashion.

10. The method of claim 6, wherein the tracing marker is conjugated to the matured dendritic cells with an antibody.

11. The method of claim 8, wherein the radionuclide is selected from the group consisting of ^{18}F, ^{32}p, ^{61}Cu, ^{90}Y, ^{99}mTc, 131, 125I 80O 111 ^{188}Re, and ^{177}Lu and the luminescence or fluorescent dye is selected from the group consisting of N, N'-di-carboxypentyl-indodicarbocyamino-5,5'-disulfonic acid (Cy5.5), Alexa Fluor probes (Alexa), carboxyfluorescein succinimidyl ester (CFSE), 4-N(S-glutathionylacetylaminophenyl)arsenoxide-Cy5.5, 2,3-dicya-nonaphthalene-Cy5.5, 4-N(S-glutathionylacetylaminophe-nyl)arsenoxide-Alexa, 4-N(S-glutathionylacetylaminophenyl)arsenoxide-CSFE, and 2,3-dicyanonaphthalene-Alexa, and 2,3-dicyanonaphthalene-CSFE.

12. A method for delivering a therapeutic agent to a site of one or more cancer cells in a subject or to a site of an autoimmune process in a subject, comprising:

contacting immature dendritic cells or monocytes with a peptide construct ex vivo under conditions suitable for maturation of the cells to form the matured dendritic cells; and

administering an effective amount of the matured dendritic cells to the subject, wherein a majority of the matured dendritic cells administered to the subject locate to the site of one or more cancer cells or the site of an autoimmune process,

wherein the matured dendritic cells are conjugated to a therapeutic agent; and

wherein the peptide construct is the peptide of claim 1.

13. The method of claim 12, wherein the immature dendritic cells or monocytes are collected from the subject, and where the cells after maturation are introduced back into the subject in an autologous fashion.

14. The method of claim 12, wherein the therapeutic agent is conjugated to the matured dendritic cells with an antibody.

15. The method of claim 12, wherein the therapeutic agent is one or more selected from the group consisting of monomethyl auristatin E (MMAE), ozogamicin, emtansine, gelonin, staphylococcal enterotoxin B (SEB) superantigen, Saporin, interferon-α, a microtubule inhibitor, an antimitotic agent, a maytansinoid, a receptor tyrosine kinase inhibitor, and a phosphoinositide 3-kinase inhibitor.

16. The method of claim 12, wherein the peptide construct is conjugated to the therapeutic agent.

17. A method for vaccinating a subject, comprising:

administering an effective amount of a peptide construct optionally with an adjuvant to the subject or administering an effective amount of matured dendritic cells to the subject, wherein the peptide construct is the peptide of claim 1; and

the matured dendritic cells are formed by contacting immature dendritic cells or monocytes with the peptide construct under conditions suitable for maturation of the cells to form matured dendritic cells,

wherein the peptide constructor the matured dendritic cells are administered to the subject prophylactically.

18. The method of claim 17, wherein the immature dendritic cells or monocytes are collected from the subject, and where the cells after maturation are introduced back into the subject in an autologous fashion.

19. The method of claim 17, wherein the peptide P_2 is selected from the group consisting of SEQ ID No's: 963 and 1024.

20. The method of claim 17, wherein the peptide construct is administered with an adjuvant that is selected from the group consisting of Freund's incomplete adjuvant, a liposomal adjuvant, a water-in-oil formulation, and a water-in-oil-in-water formulation.

21. A method of modulating an immune response in a subject, comprising: administering an effective amount of a peptide construct optionally with an adjuvant to the subject or administering an effective amount of matured dendritic cells to the subject, wherein the peptide construct is the peptide of claim 1; and

the matured dendritic cells are formed by contacting immature dendritic cells or monocytes with the peptide construct under conditions suitable for maturation of the cells to form matured dendritic cells,

wherein the peptide constructor the matured dendritic cells are administered to the subject having an autoimmune disease or condition.

22. The method of claim 21, wherein the immature dendritic cells or monocytes are collected from the subject, and where the cells after maturation are introduced back into the subject in an autologous fashion.

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